Genomic Characterization of Pleural Solitary Fibrous Tumours

by

Ghassan Allo

A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Laboratory Medicine and Pathobiology
University of Toronto

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2013

Abstract

Pleural solitary fibrous tumours (pSFTs) are uncommon soft tissue tumours of the pleura that may recur and contribute to the patients’ demise. We analyzed a group of benign and malignant pSFTs for copy number alterations and for common mutations in oncogenes and tumour-suppressor genes. Malignant SFTs demonstrated more copy number alterations, especially 8q (c-myc) gain, 10q (include PTEN) loss, and 13q (Rb1) loss. Mutations were rare in this limited study.
Acknowledgments

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>CEP</td>
<td>Chromosome enumeration probe</td>
</tr>
<tr>
<td>CGH</td>
<td>comparative genomic hybridization</td>
</tr>
<tr>
<td>CN</td>
<td>copy number</td>
</tr>
<tr>
<td>CNA</td>
<td>somatic copy number alteration</td>
</tr>
<tr>
<td>CNV</td>
<td>copy number variation</td>
</tr>
<tr>
<td>ddNTP</td>
<td>dideoxynucleotide phosphate</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxynucleotide phosphate</td>
</tr>
<tr>
<td>EMA</td>
<td>epithelial membrane antigen</td>
</tr>
<tr>
<td>FFPE</td>
<td>formalin-fixed paraffin-embedded</td>
</tr>
<tr>
<td>FISH</td>
<td>fluorescence in situ hybridization</td>
</tr>
<tr>
<td>HPF</td>
<td>high power fields</td>
</tr>
<tr>
<td>IHC</td>
<td>immunohistochemistry</td>
</tr>
<tr>
<td>LR</td>
<td>Likelihood ratio</td>
</tr>
<tr>
<td>LSI</td>
<td>locus-specific identifier</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>matrix-assisted laser desorption/ionization time-of-flight</td>
</tr>
<tr>
<td>MAPD</td>
<td>median absolute pair-wise difference</td>
</tr>
<tr>
<td>MBPs</td>
<td>million base pairs</td>
</tr>
<tr>
<td>MIP</td>
<td>Molecular inversion probe</td>
</tr>
<tr>
<td>mTOR</td>
<td>mammalian target of rapamycin</td>
</tr>
<tr>
<td>ND</td>
<td>NanoDrop</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>platelet-derived growth factor alpha</td>
</tr>
<tr>
<td>PDGFRB</td>
<td>platelet-derived growth factor neta</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homologue</td>
</tr>
<tr>
<td>PXN</td>
<td>paxillin</td>
</tr>
<tr>
<td>Rb1</td>
<td>retinoblastoma-1</td>
</tr>
<tr>
<td>SFTs</td>
<td>solitary fibrous tumours</td>
</tr>
<tr>
<td>SMA</td>
<td>smooth muscle actin</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>TKI</td>
<td>tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>TMA</td>
<td>tissue microarray</td>
</tr>
</tbody>
</table>
Chapter 1 : Introduction

1 Introduction

1.1 Solitary fibrous tumours – A Review

Solitary fibrous tumours (SFTs) are uncommon spindle cell mesenchymal tumours. They represent the most common mesenchymal tumours of the pleura\textsuperscript{1,2}. Although they were initially described to arise exclusively from pleural surfaces, they are known now to develop at various sites, but they remain far more common in the pleura and thus are primarily a disease of the pleura\textsuperscript{3}.

1.1.1 Clinical Characteristics

SFTs have been described nearly equally in both genders, with a slight male predominance; in five studies compiled, the number of males and females with SFTs was 274 (51.1%) and 262 (48.9%), respectively\textsuperscript{2,4-7}. At the time of diagnosis, the patients’ age ranged between 5 – 88 years, the majority being in the fourth to the sixth decade\textsuperscript{4,5}. Most SFTs are discovered incidentally on a routine chest X-ray or a CT scan\textsuperscript{1,8}. As they enlarge and compress adjacent thoracic structures, they may trigger cough, chest pain, dyspnea, digital clubbing, and hypertrophic osteoarthropathy. On rare occasions, SFTs produce insulin-like growth factor inducing symptomatic hypoglycemia\textsuperscript{9}.

1.1.2 Histopathologic and Ultrastructural Phenotype

The histogenetic origin of SFTs had been controversial. Historically, two main hypotheses competed to explain it. One theory postulated that SFTs originated from specialized sub-mesothelial cells, capable of differentiation to surface mesothelium\textsuperscript{4,10,11}. The other hypothesis advocated a pure mesenchymal-myofibroblastic origin\textsuperscript{12,13}. The latter hypothesis is currently
accepted, supported by detailed microscopical, immunohistochemical and ultrastructural studies, as described below.

1.1.2.1  Histopathology
SFTs are generally composed of uniform elongated spindle cells with tapered nuclei, arranged in one or more different architectural patterns\(^1, 2, 4, 14\), the most common of which is “patternless pattern”, followed by haemangiopericytoma-like. The “patternless pattern” involves spindle cells and connective tissue in haphazard arrangement and varying proportions, with alternating and juxtaposed hypo- and hypercellular areas. In contrast, haemangiopericytoma-like pattern implies closely packed tumour cells around open or collapsed irregular, branching capillaries and larger vessels. Other histological patterns include storiform, herringbone, leiomyoma-like, or neurofibroma-like.

1.1.2.2  Immunohistochemistry
Immunohistochemically, more than 90% of SFTs express CD34, CD99 and bcl-2, and lack expression of markers of muscle differentiation (smooth muscle actin), epithelial differentiation (cytokeratins, epithelial membrane antigen) and other mesenchymal markers (S-100 protein, desmin and calretinin)\(^1, 2, 15-19\).

1.1.2.3  Ultrastructural morphology
Under electron microscopy, SFTs, exhibit (myo) fibroblastic features\(^20-23\). These features include elongated spindle cells containing variably prominent rough endoplasmic reticulum, Golgi apparatus, sub-plasmalemmal thin filaments, and primitive cell junctions. Other cells are larger exhibiting some degree of smooth muscle differentiation, with more than occasional pinocytosis, and small thin filaments. Thick, irregularly arranged, distorted collagen fibrils permeate the intercellular space.
1.1.3 Prognosis

The majority of pleural SFTs are benign. Nonetheless, it has been reported that pleural SFTs could carry a local recurrence rate of 9–19% \(^2,18,21,24-26\), distant metastatic rates of 0–19% \(^2,4,18,24\), and tumour-related death rate of 0–27% of cases \(^18,21,24,25\), even with complete initial surgical excision.

1.1.3.1 Pathological Prognostic Factors

It is difficult to predict which SFTs would recur or metastasize; tumours that do so, the so-called ‘malignant’ SFTs, have been shown to harbour a number of pathological features, summarized in Table 1 \(^2-5,9,28,31-35\). These features have also been reported in regions of malignant transformation or dedifferentiation arising in otherwise benign SFTs \(^27-31\).

<table>
<thead>
<tr>
<th>Pathological features predicting malignancy in SFTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis</td>
</tr>
<tr>
<td>High mitotic count ((&gt; 4 / 10) HPF)</td>
</tr>
<tr>
<td>Hypercellularity</td>
</tr>
<tr>
<td>Moderate to marked nuclear pleomorphism</td>
</tr>
<tr>
<td>Tumour maximum dimension (&gt; 10) cm</td>
</tr>
</tbody>
</table>

Although these features are commonly used in clinical practice for SFT stratification, there are a number of factors limiting their effectiveness.

First of all, the published studies in the English literature have not been consistent in using these features; different combinations of these criteria have been employed to classify and prognosticate SFTs, as shown in the following three examples (Table 2).

In one of the largest series, England \textit{et al} classified 82 of 223 SFT cases as malignant, based on the presence of at least one of the above discussed features\(^7\). All, but two, benign SFTs were
cured with adequate surgical resection, while 39/71 (55%) patients with malignant SFTs had tumours refractory to therapy.

The second example is a study of 84 pSFT patients. The presence of 4 or more mitoses per 10 high-power fields (HPF) was the sole criterion to diagnose malignancy, while the remaining pathologic features were considered ‘supportive’ of malignant behaviour. This classification method divided the study population into malignant and benign SFTs with median survival of 55 months and 284 months, respectively, and five-year survival of 45.5% (95% CI, 24%–87%) and 89% (95% CI, 82%–96%), respectively (P = .0005).

On the other hand, malignancy was defined in a third study of 18 SFTs by Carretta et al as the presence of at least 3 of the 4 histologic features mentioned above. Although the power of this study is limited by the number of study cases, malignant histology was associated with higher recurrence and lower disease free survival (p<0.05).

Table 2: Summary of SFT studies using different malignancy criteria.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Cases</th>
<th>Criteria for Malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>England et al. 1989$^2$</td>
<td>223</td>
<td>Any pathological feature</td>
</tr>
<tr>
<td>Harrison-Philips et al. 2009$^{32}$</td>
<td>84</td>
<td>Mitoses ≥4/10 HPF</td>
</tr>
<tr>
<td>Carretta et al. 2006$^{33}$</td>
<td>18</td>
<td>3 histological features</td>
</tr>
</tbody>
</table>

Beside the variable use of these criteria by different authors, their predictive value is inconsistent; although the presence of these pathologic features correlate with malignant
behaviour in some studies, other studies and reports have failed to demonstrate this relationship\textsuperscript{34, 35}. In a study of 60 patients by Magdeleinat et al\textsuperscript{34}, SFTs were classified into benign and malignant based on the same criteria used by England et al as described above\textsuperscript{2}. However, this classification did not result in statistically different recurrence rate or survival between the 38 benign and the 22 malignant SFTs. Conversely, the presence of at least one pathological unfavorable feature was described in up to 50\% of clinically benign SFTs in several studies \textsuperscript{2, 3, 5, 24, 36}. Tumour size is one of the main features with inconsistent prognostic significance; some studies found a correlation between tumour size and behaviour, with SFTs larger than 10 cm in maximum dimension being more likely to be malignant\textsuperscript{2, 33, 37}. However, large SFTs can still behave in a benign fashion\textsuperscript{38-44}, and malignancy can still arise in small solitary fibrous tumours\textsuperscript{2, 33, 37}.

1.1.3.2 Unproven Prognostic Markers – Immunohistochemical

A Limited number of studies investigated the correlation between the expression of a number of markers in SFTs with behaviour. Below is a summary of these markers.

1.1.3.2.1 p53

p53 is a highly pleiotropic, short lived, transcription factor, which mainly functions as tumour suppressor, but is also involved in a range of physiological processes including cell metabolism, mitochondrial respiration, autophagy, cell adhesion, and stem cell maintenance and development\textsuperscript{45}. P53 cellular level varies with the physiological and the pathological status. In the absence of cellular stress, it is sustained at low levels, through degradation and transcriptional silencing mediated by MDM2 and MDM4\textsuperscript{45}. Such low level maintains the protein’s physiologic functions sufficiently, and presents as weak positivity in epithelioid cells by immunohistochemistry. Conversely, the p53 cellular level noticeably increases as a result of
oncogenic stress or damage signalling, mainly by hindering interaction with MDM2 and MDM4\textsuperscript{45}. Mutations in p53 gene also affect p53 protein expression; truncation mutation results in low expression, while other forms of mutations result in deformed p53 proteins that do not interact with MDM2, and therefore, can accumulate at high levels within the cells.

P53 cellular level can be assessed by immunohistochemistry; the physiologic short-lived low-level is weakly positive. Truncation mutations result in negative immunohistochemistry while other mutations prevent p53 protein from interacting with MDM2, and therefore accumulate intracellularly at high levels and present as strong immunohistochemical positivity\textsuperscript{46}.

Immunohistochemical p53 expression has been assessed in a total of 176 SFT cases –including 70 histologically malignant tumours\textsuperscript{5, 47-49}. The expression was associated with the presence of histological features of malignancy and poorer overall and disease-free survival in both univariate and multivariate analyses\textsuperscript{5}, whereas it is nearly absent in benign tumours. Moreover, p53 positivity has also been identified in a number of de-differentiated SFTs\textsuperscript{27-31}, emphasizing the role of deregulation of this tumour suppressor in SFT progression.

1.1.3.2.2 p16

The p16 protein is a cyclin-dependent kinase (cdk) inhibitor that decelerates the cell cycle by inactivating the cdks that phosphorylate the retinoblastoma tumour suppressor protein. Loss of p16 gene function has been reported in many human cancers\textsuperscript{50-53}. In one study, p16 immunohistochemical expression was analyzed in 15 SFTs, including 4 malignant tumours\textsuperscript{54}. In this study, p16 negativity was associated with a low recurrence rate. This pattern may be the result of cell cycle deregulation owing to inactivation or loss of other associated cell cycle regulators, such as retinoblastoma or p53 tumour suppressors\textsuperscript{53, 55}.
1.1.3.2.3 CD34

As mentioned above, CD34 is a classical phenotypic marker of SFTs, as it is expressed in the vast majority of these tumours, and in fact, is one of the markers used to confirm the histologic diagnosis of these tumours. However, a few studies, examining a total of 18 histologically malignant and 29 benign SFTs, have observed an association between lack of CD34 expression and dismal outcome of patients carrying these tumours\textsuperscript{31, 47, 48}. This pattern is reminiscent of other examples of tumour dedifferentiation, in which tumour cells demonstrate progressive loss of common histomorphologic features, and acquire a less differentiated phenotype\textsuperscript{56, 57}.

1.1.4 Treatment

Currently, complete surgical excision is the mainstay of SFT treatment\textsuperscript{2, 58}. Radiation and chemotherapy are used when resection is not optimal, or if the tumour is diffusely metastatic\textsuperscript{2, 59, 60}.

Recently, targeted therapy has been introduced in two cases of recurrent malignant SFTs of pleura that were resistant to adjuvant therapy. Tissue from the first tumour\textsuperscript{61}, and cells from the second\textsuperscript{62} were analyzed for the expression of and mutation in c-kit, platelet derived growth factor receptor alpha (PDGFRA) and beta (PDGFRB) receptors genes, which are targets of tyrosine kinase inhibitors (TKI). Both tumours expressed PDGFRA and PDGFRB, but did not show any known activating mutation in the three genes examined. Because of the diffuse metastasis and the unresectability of the tumour from the first patient, Imatinib, a multi-target tyrosine kinase inhibitor\textsuperscript{63}, was administered to this patient, resulting in clinical and biochemical tumour regression. Moreover, cells derived from the second tumour were exposed to Imatinib in vitro, resulting in a dose-dependent reduction in cell proliferation. In summary, both of these two cases uncover the potential use of molecular targeted drugs to treat SFTs. Of note, Schirosi et al have
found expression of PDGFRA and PDGFRB in 97.7% and 86.5% of SFTs, respectively (see Molecular Pathology).

1.1.5 Molecular pathology of SFTs

Cancer development is the net result of augmented cellular proliferation and deficient apoptosis, as a result of a step-wise acquisition of genetic alterations that deregulate the function of oncogenes and tumour suppressor genes, conferring growth advantage to the altered cells. These genetic alterations are now used to re-classify tumours and direct targeted management. These changes include copy-number (CN) alterations, and single base substitutions (mutations). CN alterations can either be germ-line (copy number variations – CNVs) or acquired (somatic copy number alterations – CNAs). CNAs are common in cancer, and can be recurrent with particular tumour types. They develop through a number of mechanisms, mostly related to non-allelic homologous recombination or microhomology-mediated events (homology of 2-15 bp), resulting in modified expression levels of the genes in the affected regions, including cancer genes. On the other hand, mutations, triggered by mutagens or because of erroneous incorporation during DNA replication, have a significant role in cancer development; they can be either ‘driver’ mutations, which provide growth advantages in the transformed cells and are causally implicated in cancer development, or ‘passenger’ mutations, which are pathobiologically neutral, and are transferred to the cancer from the progenitor cells that clonally expanded to form the tumour. Tumour dependence on amplified or mutant genes for their survival has recently been the focus of a number of targeted therapies. Beside the above described genetics changes, epigenetic processes, such as DNA methylation and histone covalent modification, play an important role in controlling gene expression, and hence, the oncogenic process without structural copy number changes or mutations.
Here, we discuss molecular changes that have been described in SFTs.

1.1.5.1 Conventional cytogenetics

A total of six pleural SFTs, four histologically malignant and two benign, have been examined for cytogenetic changes, and the results are summarized in Table 3. No recurrent abnormality has been identified in these cases. However, malignant SFT cases were observed to contain more abundant and complex cytogenetic alterations than benign, a phenomenon usually expected in malignant and aggressive tumours. Of these changes, abnormalities in the long arm of chromosome 9, especially in 9q21~22 region, in the form of insertions, deletions and translocation, have been observed in 3 of the 4 malignant SFTs, as reported by Donner et al, de Leval et al, and Torres-Oliviera et al. These findings suggest a potential relationship between changes around 9q and the malignant behaviour of pleural SFTs.

<table>
<thead>
<tr>
<th>Author</th>
<th>Age</th>
<th>Sex</th>
<th>Histology</th>
<th>Short Cytogenetic Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mertens et al73</td>
<td>58</td>
<td>f</td>
<td>Benign</td>
<td>46,XX,t(2;3)(p21;p26)/46,XX</td>
</tr>
<tr>
<td>Dal Cin et al74</td>
<td>67</td>
<td>m</td>
<td>Malignant</td>
<td>46,XY,t(4;15)(q13;q26)</td>
</tr>
<tr>
<td>Donner et al75</td>
<td>68</td>
<td>m</td>
<td>Malignant</td>
<td>46,XY,t(6;17)(p11;q23),ins(9;12)(q22;q15q24),inv(16)(p13q24)</td>
</tr>
<tr>
<td>de Leval et al76</td>
<td>54</td>
<td>m</td>
<td>Malignant</td>
<td>46,XY,t(1;16)(q25;p12)/48,XY,+8,+8,del(9)(q22q32)[19]/46,XY, t(1;16)(p13.1q24)</td>
</tr>
<tr>
<td>Horton et al77</td>
<td>47</td>
<td>m</td>
<td>Benign</td>
<td>46,XY,t(8;12)(p11q24)</td>
</tr>
<tr>
<td>Torres-Oliviera et al78</td>
<td>43</td>
<td>f</td>
<td>Malignant</td>
<td>47,XX,del(1)(q24),t(1;9)(q42;q34),del(9)(q21),der(9) t(9;10)(q21;p14),+der(9),del(10)(p14)</td>
</tr>
</tbody>
</table>

Age in years; f: female; m: male.
1.1.5.2 Analysis of copy number changes

No high-resolution genomic study of copy number changes in SFTs has been published yet. However, comparative genomic hybridization (CGH) was used to evaluate a total of 13 benign SFTs. Of the 12 cases examined by Krismann et al., seven (58%) cases did not show any change; the remaining five cases demonstrated more chromosomal losses than gains (see Figure 1).

![Compilation of chromosomal aberrations from the previously published 12 SFTs](image)

**Figure 1**: Compilation of chromosomal aberrations from the previously published 12 SFTs. Losses are depicted as bars on the left side of each chromosome ideogram; gains are depicted on the right side. (Source: Krismann et al 2000)

The most frequent losses in this study were in chromosome arms 13q (33%), followed by losses in 4q and 21q (17% each). Losses in chromosome 13 were reported previously in two pleural and a few extra-pleural SFTs. In addition, translocations in chromosome 13 were also reported in one intra-abdominal SFT. This highlights a potential role of chromosome 13 in SFT pathogenesis.
Different cytogenetic changes were detected on chromosome 4. These include translocations\textsuperscript{85, 86} and chromosomal loss reported in 3 extra-pleural SFTs \textsuperscript{83}. On the other hand, gains of this chromosome were reported in one pleural\textsuperscript{79}, and three extra-pleural SFTs\textsuperscript{81, 87}.

Of the chromosomal gains reported in Krissman’s study\textsuperscript{17}, gains of chromosomes 8, and of segments on 15q, were present in two cases. Chromosome 8 trisomy have also been reported by de Leval \textit{et al} in a malignant recurrent pleural SFT\textsuperscript{76}. This recurrent tumour harboured two clones: 48,XY,+8,+8,del(9)(q22q32)[19] and 46,XY,t(1;16)(q25;p12). It was cultured yielding one clone, identical to the dominant clone of the initial karyotype, with persistent chromosome 8 trisomy. Moreover, chromosome 8 trisomy has been discovered among other abnormalities in an "extra-pleural" SFT arising in the oral cavity, which appeared 'benign' on histology, and did not show evidence of recurrence after 6 years of follow up.\textsuperscript{87} In summary, gains in chromosome 8 fall among the commonly reported changes and imbalances in SFTs –both benign and malignant, an abnormality that potentially correlates with SFT development or survival.

Chromosome 15 gains have been reported in a soft tissue SFT\textsuperscript{81} and in a SFT arising around the liver.\textsuperscript{83}

In contrast, Amo-Takyi \textit{et al} have reported different copy number changes in one case of benign SFT (Figure 2)\textsuperscript{79}. In contrast to the previous study, this report showed gains on chromosomes 13 and 4, while no changes were found on chromosomes 21, 8 or 15. Additional changes include loss on 1q, 17, 19, 20q, and 22, and gain on 2q, 3, 6, and 13q.
1.1.5.3 Specific cancer-related genes

Rossi et al\textsuperscript{88} and Morimitsu et al\textsuperscript{89} studied the status of certain oncogenes and tumour suppressor genes in solitary fibrous tumours. Table 4 is a summary of their findings, classified according to the mechanism through which genes contribute to tumourigenesis. Apart from p53 (see Prognosis), none of the examined immunohistochemical markers correlated with survival.
Table 4. Summary of expression and mutation analysis results of ten genes in pleural SFTs. EGFR: Epidermal Growth Factor Receptor; PDGFR: Platelet Derived Growth Factor Receptor; Bcl-2: B-Cell Lymphoma 2

<table>
<thead>
<tr>
<th>Genes</th>
<th>Protein Expression (number of positive cases / number of cases examined)</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Factor Receptors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR</td>
<td>0/88</td>
<td>None</td>
</tr>
<tr>
<td>c-Kit</td>
<td>3/88</td>
<td>None</td>
</tr>
<tr>
<td>PDGFR-beta</td>
<td>76/88</td>
<td>Missense mutation in exons 18 (n=1) and 20 (n=1)</td>
</tr>
<tr>
<td>PDGFR-alpha</td>
<td>86/88</td>
<td>None</td>
</tr>
<tr>
<td>c-Met</td>
<td>85/88</td>
<td>None</td>
</tr>
<tr>
<td><strong>Signal Transduction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>Not reported</td>
<td>None</td>
</tr>
<tr>
<td><strong>Apoptosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl-2</td>
<td>18/18</td>
<td>Not reported</td>
</tr>
<tr>
<td><strong>DNA Repair</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P53</td>
<td>*</td>
<td>Point mutation at codon 161 of exon 5 in one case</td>
</tr>
<tr>
<td>Mdm-2</td>
<td>0/15</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

* Positive in malignant component

In summary, a limited number of studies have explored the molecular mechanisms that underlie the development and progression of pleural SFTs. In the published studies, the reported changes were not common. High-resolution genome-wide analysis of these tumours has not been performed.
1.2 Methods in Tumour Genomics – a Focused Review

1.2.1 Tumour Samples – Hurdles and Challenges

Biobanks provide a good source of tissue for high-quality nucleic acid and protein extraction, and have helped advance molecular research\textsuperscript{90}. However, biobanked frozen material remains limited in quantity and entails a number of challenges. Nevertheless, archival formalin-fixed paraffin-embedded (FFPE) tissue continues to be widely available and an invaluable source for research, especially for rare tumours, such as SFTs, that may not be sufficiently banked for research purposes.

Fixation in formalin and subsequent embedding in paraffin has been used for decades to prepare tissue for histopathological examination and subsequent storage. This archived tissue can be promptly employed for morphological studies, for immunohistochemical protein expression and for in-situ hybridization. However, nucleic acids extracted from archived tissue are generally of poor quality, because treatment with formaldehyde and embedding in paraffin results in nucleic acid fragmentation (down to 300 base pairs in length) and cross-linking with proteins\textsuperscript{91}. This low quality of nucleic acids imposes a great challenge to the application of genomic-wide technologies on FFPE samples, leaving only a handful of technologies and platforms usable to analyze this form of tissue at the present time\textsuperscript{91}. Below is a review of the technology behind some of the methods that perform well with low-quality FFPE samples.

1.2.2 Copy Number Analysis

1.2.2.1 OncoScan™ FFPE Express 330K MIP platform

This platform, offered by Affymetrix (Santa Clara, CA)\textsuperscript{92, 93}, allows a high-resolution copy number analysis of a variety of samples, including FFPE-derived DNA. Following is a description of the platform’s technology, advantages and limitations.
1.2.2.1.1 Technology - Molecular Inversion Probe

This platform exploits a fairly novel technology called the molecular inversion probe (MIP) \(^{92,93}\). The MIP is an oligonucleotide composed of seven segments (Figure 3.A)\(^ {94,95} \): Firstly, there are two regions homologous to a target on the genomic DNA. These homologous regions are probe-specific, and are located at each terminus of the probe. In addition, there are two PCR primer regions common to all probes, two common cleavage sites, and one locus-specific bar code (tag) region that has the same melting temperature and base composition as other probes.
Figure 3. Molecular inversion probe reaction (Adapted from Hardenbol et al 2003, and http://www.ncbi.nlm.nih.gov/projects/genome/probe/doc/TechMIP.shtml)
The reaction takes place by mixing genomic DNA under study with distinctly designed molecular inversion probes, together with thermostable ligase and polymerase. When this mixture is brought to an annealing temperature, the two homologous regions at the opposite ends of the linear probe hybridize to a complementary region on the genomic DNA in such a way that the probe becomes circularized, but leaving a single-nucleotide gap between the termini of the probe unfilled (Figure 3.B). Then a gap-filling step takes place by adding the four forms of deoxynucleotide phosphate (dNTP) to the genomic DNA-hybridized probe reaction (Figure 3.C and 3H). The presence of a dNTP complementary to the nucleotide in the gap leads to filling this gap and closing the probe circle, by the action of polymerase, and ligase respectively. Non-circularized probes, resulting from non-complementary added nucleotides, and excess linear probes are degraded by adding exonucleases, which can be inactivated by heating in the due course (Figure 3.D). Heating will release the probe from genomic DNA and restriction enzymes will cleave the circularized probe at one of the cleavage sites between the primers (Figure 3.E). The released probe, as compared to the original probe, is inverted in such a way that the homologous regions are joined in the middle of the probe, and the PCR primer sites at the termini (Figure 3.F). Next, PCR using the common probe primer site results in the amplification of only the probes that were circularized in the allele-specific gap-filling reaction (Figure 3.G). Amplified probes are then hybridized to annotated DNA microarrays according to their probe-specific tags (bar codes), and the components are decoded by measuring the fluorescence signals at the corresponding complementary tag site on the DNA array (Figure 3.H). Genotype at each locus is then called after proper background subtraction, colour separation, and normalization.

1.2.2.1.2 Specifications and Advantages of OncoScan platform

The specifications and marker distribution of OncoScan array are summarized in Table 5. This platform entails a number of advantages that make it suitable for studying DNA extracted from
FFPE tissue\textsuperscript{93, 94, 96-98}. Firstly, FFPE-induced fragmentation and cross-linking is overcome to a significant level as hybridization in the MIP reaction occur at a short length of the genomic DNA, being equal to the length of the probes’ homologous regions (40 base pairs per probe). Secondly, the amplification only takes place on the successfully hybridized and circularized probes. Amplification using universal primers and conditions for all probes is predictable and uniform, unlike whole genome amplification using genomic DNA which is affected by the quality of the DNA itself. Controlled amplification using MIP results in generally consistent representation of the randomly fragmented DNA on the platform. Thirdly, this platform has been optimized to yield high-resolution copy number data using only low amounts of genomic DNA (37-75 ng), while maintaining a broad dynamic range. It can be customized and multiplexed for specific regions of interest instead of the pre-designed platform already offered by the company.

The MIP platform has been validated for allele quantification and copy number analysis on new and old archival samples\textsuperscript{92, 93}, and has been used to detect copy number changes in leukemia\textsuperscript{99}, and in breast\textsuperscript{100}, ovarian\textsuperscript{101, 102}, and colorectal neoplasms\textsuperscript{103}. 
Table 5: Marker distribution of OncoScan FFPE Express v.1.0 array

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Number of Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of copy number markers</td>
<td>330,007</td>
</tr>
<tr>
<td>Number of non-polymorphic markers</td>
<td>16,776</td>
</tr>
<tr>
<td>Autosomal markers</td>
<td>329,984</td>
</tr>
<tr>
<td>Pseudoautosomal markers</td>
<td>23</td>
</tr>
<tr>
<td>Intragenic markers</td>
<td>164,826</td>
</tr>
<tr>
<td>Intergenic markers</td>
<td>165,181</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Functional category</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Conserved regions</td>
<td>32,935</td>
</tr>
<tr>
<td>cSNPs</td>
<td>18,748</td>
</tr>
<tr>
<td>UTR</td>
<td>5,483</td>
</tr>
<tr>
<td>Splice sites</td>
<td>59</td>
</tr>
<tr>
<td>chrX</td>
<td>7,428</td>
</tr>
<tr>
<td>MHC</td>
<td>115</td>
</tr>
<tr>
<td>ADME</td>
<td>5,202</td>
</tr>
<tr>
<td>chrY</td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Median marker spacing</th>
<th>Base pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intragenic markers</td>
<td>2,872</td>
</tr>
<tr>
<td>RefSeq genes</td>
<td>2,872</td>
</tr>
<tr>
<td>Cancer genes</td>
<td>2,127</td>
</tr>
<tr>
<td>Cytogenetic relevant/haploinsufficiency genes</td>
<td>2,722</td>
</tr>
<tr>
<td>X chromosome genes</td>
<td>3,140</td>
</tr>
<tr>
<td>OMIM genes</td>
<td>2,610</td>
</tr>
<tr>
<td>Intergenic markers</td>
<td>5,067</td>
</tr>
<tr>
<td>Overall median marker spacing</td>
<td>4,208</td>
</tr>
<tr>
<td>Average marker spacing (base pairs)</td>
<td>9,119</td>
</tr>
</tbody>
</table>

1.2.2.1.3 Limitations

OncoScan platform shares a number of limitations with other copy number array-based technologies (array comparative genomic hybridization and single nucleotide pleomorphism arrays). First of all, OncoScan copy number analysis is derived from the absolute imbalance of DNA dosage between a disease sample (such as cancer) and a control sample. Accordingly, events that do not result in copy number imbalances, such as balanced translocations and
inversions, cannot be identified. Moreover, the net copy number imbalance generated by these arrays does not explain the mechanisms that underlie the copy number alterations; therefore, the platform would not differentiate between an unbalanced translocation, a duplication, an insertion, or a marker chromosome. Additional studies, such as florescence in-situ hybridization may be used to reveal these mechanisms should they be suspected. One other limitation is that the findings depends on the selection and the distribution of probes or assays; hence, under-represented areas may falsely be considered to have normal DNA copy numbers, and over-representation may result in over-estimation of genomic imbalances. Although the number of OncoScan markers covering intragenic and intergenic regions, stated in Table 5, is almost the same, markers are more concentrated in the former regions, which are presumably of more biological relevance. Additionally, these probes cover known cancer-related genes in higher density than other regions.

1.2.2.1.4 Bioinformatics

Analysis of OncoScan CN data, like other platforms, requires data quality control and probes clustering, followed by CN inference of each probe cluster. These steps can be carried out manually or through the use of proprietary software. Affymetrix recommends the use of Nexus Copy Number™ v.6.0.beta (BioDiscovery, Howthorne CA) software package. Following is a description of how this software handles the copy number data.

Data quality control is attained at multiple levels. Firstly, the median absolute pair-wise difference (MAPD) is used to assess and eliminate cases with high noise level\(^93\). The MAPD is the median of the \(\log_2\) differences between neighboring probes for all pairs of probes on the array for an entire sample, representing the typical distance between marker pairs with respect to \(\log_2\) ratio. The higher the MAPD, the greater the noise between the sample and the reference set is.
For the OncoScan study, a MAPD threshold value of less than or equal 0.6 is considered a pass. Among the valid cases, only probes with call rate of 90% or greater and relative standard deviation of less than 30% are considered valid for the analysis. At the probe level, outlier probes that significantly differ from adjacent probes are eliminated, first by calculating the absolute differences between adjacent probes, then by ordering these values by magnitude, followed by removing 3% of the probes that fall at the top and bottom of the rank list. To further reduce the noise resulting from individual probe values, probes are clustered into segments according to their chromosomal locations and their log₂ values using a proprietary segmentation algorithm called the single nucleotide polymorphism fast adaptive states segmentation technique (SNP-FASST2). This algorithm is based on the well-known Hidden Markov Model (HMM) approach, after modifications to reduce the effect of restrictions that HMM relies on. It is a recursive algorithm that keeps dividing the genome into smaller and smaller segments until no region can be further segmented. During segmentation, the probes log ratio values in each region are ranked and their distribution is compared to that of probes in adjacent regions. If these distributions are significantly different, based on a pre-determined ‘Significance Threshold’, the segment is further divided. Segmentation continues until no segment can be found to be significantly different from its neighbors. The Significance Threshold is determined based on the number of assay probes. Other adjustable parameters include the minimum number of probes required to create a segment, and the maximum spacing between adjacent probes before breaking a segment. Copy number is inferred by obtaining the median log-ratio value of all probes within a segment. A segment with a log-ratio value of zero is regarded to have a normal copy number. Thresholds for CN gain, high gain, loss, and big loss can be tuned according to the platform. For OncoScan, the thresholds are set to 0.3, 1.2, -0.3, and -1.2, respectively, allowing for the
potential inaccuracy resulting from the imperfect performance of the array and the low-quality genomic DNA\textsuperscript{93}.

Areas with CN variations (CNVs) may not be real players in cancer development, and should be identified. Regions with copy number abnormalities are compared to online CNV databases\textsuperscript{105, 106}, and the areas rich in CNVs are excluded. Non-CNV altered regions are then analyzed for the presence of previously reported oncogenes and tumour suppressor genes\textsuperscript{107, 108}.

Genomic instability in each of the tumours can be assessed by calculating the fragment of genome altered (FGA), by obtaining the percentage of the length of altered genome using the OncoScan data, to the total length of the genome (3,107,677,273 bases) according to the NCBI36/hg18 genome build\textsuperscript{109, 110}. FGA of different sample groups is compared using the non-parametric Mann-Whitney U test\textsuperscript{111}. Non-parametric tests eliminate or significantly minimize the need for making assumptions regarding the data (e.g. normal distribution), and are therefore better than parametric tests (such as student t-test) especially in a small sized study cohort\textsuperscript{112}.

1.2.2.2 Fluorescence in situ Hybridization (FISH)

Interphase FISH is a widely used method to visualize chromosomal loci within intact nuclei using fluorochrome-labelled probes\textsuperscript{113}. The probes can target a repeated sequence, can label centromeres that are usually used in chromosomal numeration and copy number analysis, or they can be complementary to a unique sequence covering a chromosomal locus of interest. Depending on the study and the size of the target region, probes can be either whole chromosome paintings, partial chromosome paintings (covering large chromosome regions), yeast artificial chromosomes (200-2000 Kbp), bacterial artificial chromosomes (up to 300 Kbp), or cosmids (30-50 kbp)\textsuperscript{113, 114}. FISH can be applied on a variety of specimens including FFPE tissue and cytology specimens\textsuperscript{115, 116}.
1.2.2.2.1 Uses and Advantages

FISH allows the identification of numerical and structural chromosomal anomalies in a sensitive and specific manner\textsuperscript{113}. By comparing the number of signals from a probe, which targets a specific gene region or chromosomal locus, to the number of signals from a control region (usually a centromere), the copy number of the chromosomal region of interest can be deduced. On the other hand, using probes that target different ends of the same gene, or using probes that target two known genes simultaneously, and then examining the location of the two different signals can reveal structural anomalies such as translocations. Another advantages of FISH is that it allows direct visualization of the cellular and nuclear morphology of the studied tissue, and consequently allowing for the selection of cells to analyze (e.g. only cancer cells and not non-neoplastic stromal cells). This in turn allows the identification of abnormalities in a subset of cells, and yield results with specificity comparable to that obtained from DNA-based PCR or comparative genomic hybridization using more tedious DNA extraction methods such as laser capture micro-dissection\textsuperscript{113}. Finally, as it can be applied to tissue of a variety of types that was obtained by a range of methods such as FFPE tissue and cytology specimens, FISH represents one of the readily available and feasible methods to investigate specific chromosomal regions for anomalies in archival tissue obtained from samples of clinical and biological relevance\textsuperscript{113}.

1.2.2.2 Limitations

A few issues should be considered when designing a FISH experiment. Designing and preparing probes can be complex as it requires specific customization to target the specific chromosomal region of interest, e.g. an assay might yield a false negative result if the probe targets a part of the gene that is not altered, missing the altered segment. An additional disadvantage is that FISH is not high-throughput because it cannot be multiplexed, and counting signals can be time-consuming.
1.2.2.2.3 Scoring and Bioinformatics

For CN analysis, FISH hybridization signals of both the target and the control probes are enumerated in intact non-overlapping tumour cells. The number of cells to be counted should be enough to produce statistical confidence in the results, and a minimum of 50-100 cells should be assessed.

The thickness of the tissue used for FISH is 5 µm. As a result, sectioning can result in truncation of the nuclei. Therefore, the chromosomes under study may not be fully represented on the FISH tissue section, and the estimated copy number is inaccurate. This effect is particularly important if the aim of the study is to find whole-chromosome monosomies. However, it is assumed that this truncation and misestimation of the copy number occur almost equally in both tumour and non-neoplastic normal tissue, whose cells are of similar size and shape to the tumour cells. While tumour cells are generally characterized by genomic instability, non-neoplastic normal tissue is presumed to contain normal copy number profile. For these reasons, the truncation effect can be reduced by performing the same FISH assay on normal tissue whose cells have the same size and shape as the tumour cells, and calculating the average of cells that artifactually show abnormal signal ratios, and then using that to develop a threshold for copy number changes in the tumour tissue. This normal control tissue is usually benign tissue of the same line of differentiation as the tumour of interest. In the case of pleural SFTs, the perfectly matching benign counterpart does not exist; while tissue fibroblasts may mimic SFT cells, they are relatively smaller than these tumour cells, especially from malignant ones. In the absence of a proper non-neoplastic control, the truncation effect in our confirmatory FISH is minimized by limiting the analysis to tumour cells which contained at least 2 centromeric control signals. While this method potentially eliminates a significant proportion of truncated nuclei, its drawback is that the assessment is based merely on deviation of the target signal count from the CEP control signal.
count, limiting its ability to evaluate for monosomies involving an entire chromosome, or to
discover specific deletions that occur in a background of a chromosomal monosomy.

After obtaining signal count, CN can then be inferred using pre-determined thresholds based on
the average ratio of target:control signal ratio in a sample (ratio of ≥2 denotes gains and that ≤0.5
denotes losses). The number of cells with a particular CN change reflects the extent of that
abnormality, and the absolute signal count mirrors its magnitude.

1.2.3  Mutation Analysis
1.2.3.1  OncoScan™ FFPE Express 330K MIP platform
Beside copy number data, the OncoScan MIP platform provides information on 412
assays/mutations on 46 genes (Figure 4). OncoScan mutation analysis is based on the assumption
that mutations represent rare alleles. Consequently, signals from assays of wild-type genotype
would cluster together if plotted against a contrast value (a value that measures the separation of
allele intensities into three clusters in a single dimension: WT/WT, WT/mut, and mut/mut)\textsuperscript{118}. A
somatic “mutation score” for each assay is then generated for each sample, measuring how far
this assay is from the wild-type cluster. The larger the score is, the farther the assay is from the
cluster. Currently, a cut-off mutation score of 9 indicates a mutation for all the mutation assays,
except for BRAF_pV600E mutation assay in which a score of 4 indicates a valid mutation. As
this score is mutation and sample dependant, validation of discovered mutations with another
method (e.g. sequencing) should be undertaken.
Figure 4: Genes screened for mutations, arranged alphabetically, and the number of assays per gene using the OncoScan and the custom mass spectrometry panels.
1.2.3.2  Sequenom™ MassARRAY® System

1.2.3.2.1  Technology – Single Allele Base Extension Reaction and MALDI-TOF Mass Spectrometry

The Sequenom™ system employs the use of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) for DNA analysis \(^{119-121}\). This method is based on two general principles: firstly, DNA strand elongation can be terminated when dideoxynucleotide phosphate (ddNTP) is added to DNA chain instead of the normal deoxynucleotides phosphate (dNTP); secondly, the kinetic energy of DNA fragments (and hence the speed at which they travel) when they are boosted with energy is related to their mass (and length). Below is a description of how the assay is performed\(^{119}\).

Initially, regions of genomic DNA spanning mutations of interest are selectively amplified. After product clean-up, single base primer extension reaction is carried out by annealing selective primers to regions near the location of mutations to be analyzed, and elongation of the DNA chain by adding dNTPs and ddNTPs. The reaction is designed in such a way that DNA chain extension is terminated when a fragment contains the mutant allele but not the wild-type allele, resulting in different length of fragments based on the presence of the mutation. This difference is detected using MALDI-TOF MS, which is done by mixing the generated DNA fragments with a matrix solution (Figure 5)\(^{119}\), and then spotted on to a target plate and allowed to crystallize. The resulting crystal is hit with a laser to ionize the analyte and introduce it into the flight tube. These ions are then made to pass through an electric field, which causes them to fly through the flight tube to the detector, the MassARRAY®. Ionized DNA fragments travel at a speed dependant on their molecular weight; lighter ions (smaller DNA fragments) travel faster than heavier ions (larger DNA fragments), leading to a separation of fragments in the flight tube.
based on their mass difference. The time of flight is measured and a calibration factor is used to convert this value into the mass-to-charge ratio (mass spectrum). As each nucleotide has a different mass, the presence of a spectral peak at the site of the mutated allele confirms the presence of the mutation. This can also be designed to detect frame shift mutations (as a result of insertions and deletions) by multiplexing assays to detect the presence of duplicate peaks at alleles distant to the site of the frame-shift mutation\textsuperscript{119,121}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Schematic representation of MALDI-TOF mass spectrometry for DNA analysis. Crystallized DNA molecules are ionized with a nitrogen laser, producing DNA ions that pass through a flight tube towards a detector. (Source: Edwards et. al, 2005)}
\end{figure}

\subsection{Advantages}

Sequenom MassARRAY technology is a better mutation screening tool than traditional sequencing methods such as Sanger sequencing and pyrosequencing, for a number of reasons. Firstly, this technology has been reported to have at least the same sensitivity in detecting common mutations, and even better capability in detecting low-level (as low as 0.5\%) and frame-
shift mutations when compared to the other sequencing methods$^{119-122}$. Furthermore, assays in MassARRAY system can be highly multiplexed (up to 15 assays per well) and optimized to analyze a 384-element chip array within an hour, and genotype at a rate of 5760 genotypes/hour$^{95,96,98}$. As a result, this technology is significantly time and cost effective, and requires less DNA than other sequencing methodologies. Furthermore, the assays can be selected to target specific genes or pathways understudy. Finally, this technique has been automated, to perform accurately, consistently and efficiently.

1.2.3.2.3 Limitations

One major limitation of the MALDI-TOF MS is its inability to maintain a high efficacy when it is used to sequence long DNA reads (more than 25 base pairs) as larger DNA molecules break up more strongly in MALDI, decreasing the sensitivity nearly exponentially$^{123}$. For the mutation analysis assay, this drawback can be avoided by designing the base extension reaction product to be not more than 25 base pairs in length. Other potential limitations include primer dimerization and the formation of a false spectral peak, and the premature termination of the base extension reaction by the early separation of the primer from the template DNA$^{119}$. Careful primer designing, amplification and extension products cleaning, and rigorous confirmation of findings can help prevent and identify such limitations. Finally, mutation detection with this technology requires pre-knowledge of the specific mutations, and their use in novel mutation discovery is limited.

1.2.3.3 Sanger Sequencing

Sanger sequencing, also known as the irreversible chain termination method, was developed by Sanger and Coulson in the late 1970s$^{124}$, and is considered the “gold standard” method for sequencing and small-scale mutation detection$^{125}$. 
The main principle behind this method is the irreversible termination of DNA synthesis by means of dideoxynucleotides (ddNTPs) \(^{114, 124}\). These ddNTPs, when included in a DNA synthesis reaction, incorporate into the growing chain of DNA but block further DNA synthesis because of lack of the hydroxyl group on the 3’- position of the deoxyribose.

DNA synthesis in Sanger sequencing requires a purified DNA template, usually produced by one or more rounds of PCR amplification of genomic DNA around the sequence of interest. Synthesis of a complementary strand is carried out by mixing the denatured single-stranded PCR product with a specific primer, a DNA polymerase enzyme, and each of the four deoxynucleotides. In addition, ddNTPs, differentially labelled with a fluorescent marker at the 3’- end, are included in the reaction. As a result, four distinct families of DNA strands are produced each ending in one of the four ddNTPs. By running the newly generated DNA strands through a capillary electrophoresis system, and detecting the different fluorescent signals, the nucleotide sequence can be deduced based on the length of the each of the DNA strands and the ddNTP that it carries.

Sanger sequencing experiments are generally easy to design and perform, and do not require extensive bioinformatic analysis, and with automation, can be run with high efficiency and accuracy. However, sequencing with this method does have its limitations\(^{125}\). Firstly, this technique is low-throughput, and it is very costly and time consuming to sequence the whole genome using Sanger sequencing as compared to the more modern next generation sequencing technologies. Furthermore, there are a number of factors that may affect its specificity, including non-specific binding of the primers to the DNA, the presence of repeats in the region surrounding the sequence of interest, and pre-mature strand synthesis termination because of the presence of DNA secondary structure. Some of these limitations may be minimized by
meticulous primer design and *in silico* validation. Finally, Sanger sequencing is limited to examining regions bounded by known DNA sequences, unless a vector is used instead at the primer site\textsuperscript{125}.

### 1.3 Hypothesis

We hypothesize that genome-wide changes exist in pleural solitary fibrous tumours, and that these changes correlate with the pathological phenotype and the clinical course of these tumours.

We aim to examine a group of pleural solitary fibrous tumours for the presence of genome-wide copy number alterations, and to screen them for the presence of known mutations in cancer-associated genes.
Chapter 2: Experimental Design, Materials and Methods

2

2.1 Materials and Methods

2.1.1 Patients

After obtaining an approval from the University Health Network (UHN) Research Ethics Board to conduct this study, the pathology archives at the department of Anatomic Pathology, Laboratory Medicine Program at UHN were searched for consecutive cases of pleural solitary fibrous tumours that were resected between January 2000 and July 2010. Clinical records were reviewed, and histopathology was evaluated, specifically assessing for necrosis and the number of mitoses per 10 high-power microscopic fields (HPF). Because of the scarcity of SFTs, only FFPE tissue samples were available, which were used for DNA extraction for microarray analyses and validation, and for tissue microarray construction and immunohistochemical studies.

2.1.1.1 DNA extraction and quality control

Two non-necrotic, tumour-rich areas and non-tumour areas (histologically normal lung or lymph node tissue) were selected from each case. These areas were selected to be histologically variable, when applicable, to overcome potential tumour heterogeneity. Cores from each of these areas were obtained using a 1-mm core needle. Genomic DNA was isolated from these cores through series of deparaffinization, tissue digestion, organic extraction, precipitation and washings, as follows\textsuperscript{126,127}. First, tissue cores were deparaffinized using successive washes with xylene and ethanol alcohol. Next, tissue digestion and polypeptide degradation were achieved using overnight treatment with proteinase K. Organic extraction was then performed using 25:24:1 phenol:chloroform:isoamyl alcohol; while phenol dissolves proteins, chloroform
dissolves and denatures cell membrane lipids and separates the solution into aqueous phase (containing nucleic acids) and organic phase (containing lipids and proteins). Isoamyl alcohol prevents foaming of the solution and aids in the separation of the organic and aqueous phases. Afterward, DNA precipitation and purification from contaminating salts was performed using 3-molar sodium acetate and pelleting by centrifugation at 12,000 xg for 2-3 times. Finally, successive DNA washing with ethanol was carried out.

DNA was quantified using NanoDrop ND-1000 spectrophotometry (Thermo Scientific, Wilmington, DE) by measuring the nucleic acids’ UV absorbance at 260 nm. Absorbance of a chromophore, such as nucleic acids, correlates with the concentration of that chromophore according to the Beer-Lambert Law \( A = ebC \), where \( A \) is the absorbance at a specific wavelength, such as A260 for nucleic acids, \( e \) is the molar extinction coefficient of the chromophore, \( b \) is the path length, and \( C \) is the concentration. According to this law, path length is the only adjustable factor in this correlation. Therefore, assessment of nucleic acids at determined optimal path lengths (1-mm, 0.2-mm, 0.1-mm, 0.05-mm) by NanoDrop provides an extensive concentration measurement range (2 ng/μl to 15,000 ng/μl double stranded DNA). However, absorbance-based method has a number of disadvantages, mainly the contribution of nucleotides, single-stranded nucleic acids, organic solvents and proteins to the absorbance value, and its inability to distinguish between DNA and RNA. This contaminant-induced increase in absorbance primarily occurs at wavelengths of 280 nm and 230 nm. Therefore, examination of the ratio of absorbance at 260 nm to the absorbance at 280 and 230 (A260/A280 and A260/A230, respectively) can estimate the degree of purity of the nucleic acid sample; samples yielding ratios of 1.8 are considered “pure”, and those yielding ratios of 1.5 are considered acceptable.
In addition, the quality of the extracted DNA was assessed by staining both the original genomic DNA, and its whole-genome amplification product with ethidium bromide and electrophoresing and visualizing them on 1% agarose gel\(^{114}\). This method allowed the assessment of the integrity and the size of the extracted nucleic acid by comparing its staining pattern to that of a control DNA ladder. Samples considered suitable for further genomic analysis were those with genomic DNA larger than 1 kilo base-pairs, and those with amplified DNA forming appropriate bands on the gel electrophoresis.

2.1.1.2 Tissue Microarray (TMA) construction

A TMA was constructed as described before\(^ {132}\). First, representative areas rich in viable and cellular SFT were annotated on hematoxylin and eosin-stained slides, and on corresponding FFPE blocks. These areas were selected to encompass histologically different areas within a tumour when applicable. Two cores measuring 1.0 mm in diameter were drilled from the blocks. The cores were then mounted in linear arrays on a paraffin TMA block, according to a pre-designed map.

2.1.2 Copy Number Analysis

2.1.2.1 OncoScan™ FFPE Express 330K MIP platform

High-resolution copy number analysis of SFTs was performed using OncoScan™ FFPE Express 330K MIP platform (Affymetrix, Santa Clara, CA). Tumour and non-tumour samples were processed by Affymetrix™ laboratory, which was blinded to the clinicopathological features of the tumour samples.

2.1.2.2 Fluorescence in situ Hybridization (FISH)

In this study, FISH was used to validate the copy number calls identified on the OncoScan MIP array for selected relevant genes.
FISH was performed as described earlier\textsuperscript{115}. Briefly, FFPE Tissue sections of the TMA, each 5-μm-thick, were used. These sections were first deparaffinized with xylene washes, and dehydrated with ethanol. Air-dried slides were incubated in 10 mM citric acid buffer (pH=6.0) at 80°C for 30 min, then in 2x standard saline citrate (SSC) at pH=7.2 for 5 min at room temperature. Tissue was then digested with 37,000U pepsin in 0.01N HCl (Sigma) for 20 min at 37°C, and washed with distilled water and ethanol. FISH assays were performed using the following probes: for PTEN, LSI PTEN and CEP10 (Abbott Molecular, Mississauga ON); for c-myc, LSI C-C-MYC Dual Color with CEP12 (Abbott Molecular, Mississauga ON); and for RB1, LSI D13S319 and CEP12 (Abbott Molecular, Mississauga ON). After adding the probes to the treated slides, cover-slipping and sealing, co-denaturation was carried out at 80°C for 10 minutes in a microprocessor-controlled system (Hybrite; Vysis Inc.), followed by overnight hybridization at 37°C. Post-hybridization washes consisted of 2xSSC/0.3% NP40 at 72°C for approximately 2 minutes followed by 2xSSC at room temperature for 2 minutes. Finally, the slides were air dried in a dark chamber, and underwent chromatin counterstaining with 40,6-diamidino-2-phenylindole (DAPI) (0.15 mg/mL in Vectashield mounting medium; Vector Laboratories, Burlingame CA).

The analysis was performed blinded to the OncoScan copy number and clinical data using AxioImager System fluorescence microscope (M1 Zeiss, Gottingen, Germany) and the Isis color fluorescence and FISH imaging system (MetaSystems, Germany). Hybridization signals from the target and control probes were enumerated in nuclei of a minimum of 50 non-overlapping intact SFT cells. Cases with fewer assessable tumour cells were excluded from the analysis. Only cells with two centromeric signals were included in the CN analysis.
2.1.3 Mutation Analysis

OncoScan MIP array, and a custom-designed mass spectrometry-based Sequnom™ array were used to screen SFTs for the presence of known mutations in cancer-related genes. Samples showing potential mutations on any platform were re-analyzed to confirm their presence using Sanger sequencing of the region around these particular mutations.

2.1.3.1 OncoScan™ FFPE Express 330K MIP platform

The OncoScan mutation score was used to infer the presence of mutation. A threshold was used according to the service provider’s recommendation; an assay with a score of 9 or more is considered a potential mutation, except for BRAF_pV600E mutation assay in which a score of 4 indicates a valid mutation.

2.1.3.2 Sequenom™ MassARRAY® System

SFTs were screened for the presence of mutations in cancer related genes using a custom-designed Sequenom™ MassARRAY® platform. We have designed a custom Sequenom™ MassARRAY® panel, that screen for mutations commonly reported in lung cancers. These mutations were retrieved from the Sanger Institute Catalogue of Somatic Mutations in Cancer web site, http://www.sanger.ac.uk/cosmic. We identified 144 mutations from 17 genes (Figure 4), and multi-plexed them into 114 assays (PCR primers and extension probes) using MassARRAY® Assay Design 3.1 software (Sequenom, San Diego CA). The experiments were carried out using 20 ng of genomic DNA. Briefly, the DNA was amplified through a PCR reaction, followed by deactivation of unincorporated dNTPs using shrimp alkaline phosphatase (SAP). A single base pair extension reaction was performed by means of iPLEX® Pro buffer plus, extension probes, and thermosequenase enzyme. The products of the primer extension reaction were purified and loaded onto SpectroCHIP® Arrays. Mutant and wild-type alleles were
determined via mass spectrometry using the Sequenom MassARRAY Analyser 4 software. Mutant allele peaks of at least 10% were considered mutations after manual review.

2.1.3.3 Sanger Sequencing

In our project, we aimed at confirming the presence of mutations that were detected by the screening methods. As the OncoScan platform yielded a high number of potentially mutated assays, we selected only the assays most frequently mutated for further validation with sequencing. All potential mutations detected by the custom mass spectrometry panel (which were few) were re-analyzed by Sanger sequencing.
Table 6 lists the assays selected for sequencing, and the primers used for amplification and sequencing. These primers were designed and validated in-silico using Primer3 (http://frodo.wi.mit.edu/)\textsuperscript{135} and jPCR\textsuperscript{136}. PCR products were sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit (version 3.1, Applied Biosystems) and an ABI Genetic Analyzer (model 3100, Applied Biosystems). Sequence data were analyzed by means of SeqScape software v.2.1.1 (Applied Biosystems), followed by manual review with Chromas Lite software v.2.01 (Technelysium Pty Ltd). Allele variations that were present in both directions in more than 15 percent of tumour DNA were considered positive.
<table>
<thead>
<tr>
<th>Assay</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
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<td>CAGTGGTGAGATGCTGCAA</td>
</tr>
<tr>
<td>APC_pR332X_c994C_T</td>
<td>TTCAATTGTTGCTAATGCTCTGG</td>
<td>CAGCTGTCTTGAGGAGCTAGA</td>
</tr>
<tr>
<td>BRAF_pG466E_c1397G_A</td>
<td>TCCCTCTCAGGCTAATGCTAAGTAA</td>
<td>CGAACAGTGAGATATTCCCTTGTATG</td>
</tr>
<tr>
<td>BRAF_pV600E_c1799T_A</td>
<td>AATTCTTGTCTGATAGGATAAATGA</td>
<td>TGACTTTCTAGTAACCGACAGCATC</td>
</tr>
<tr>
<td>CSF1R_pL301S_c902T_C</td>
<td>GAAATCGCAGATCTTGTGTTCTGTC</td>
<td>GTCGACGTCACCTTGGATGTGAGTT</td>
</tr>
<tr>
<td>EGFR_pD761Y_c2281G_T</td>
<td>GTGCTTGCTCTAGAAGAAAAATGA</td>
<td>TTCTAGTAACCTCGAGCATC</td>
</tr>
<tr>
<td>KRAS codon 12/13</td>
<td>GTGGAGTATTTGTAGTAGTATCTTTAC</td>
<td>TGTATCAAAGATGGTCTGCA</td>
</tr>
<tr>
<td>MET_pE168D_c504G&gt;T</td>
<td>CAGAGGAGACTGCCAGAGA</td>
<td>TGGTCCGTCAAAAACAAAACC</td>
</tr>
<tr>
<td>MET_pN375S_c1124A_G</td>
<td>CAGAAAAGAGAAAAAGAGATCCCAAAAA</td>
<td>TGAATGGTGGTCCGTTAAAAATGC</td>
</tr>
<tr>
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<td>CCCTCTCCCGTCTGGGTGTCG</td>
<td>CAGGAGACAGCTGCCAGAA</td>
</tr>
<tr>
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<td>CCTAGCAACCTGACAGGGTTAAATG</td>
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<tr>
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<td>TCCCTTTGAGTGGTCGAC</td>
</tr>
<tr>
<td>PXN_pA127T_c379G_A</td>
<td>GCCTTGTCTCTCTGGAATCTG</td>
<td>TAGAGGTACAGATGGGCCCCG</td>
</tr>
<tr>
<td>RB1_pR552X_c1654C_T</td>
<td>ACGTGGTGAGGCAAGGGATCCATGATCG</td>
<td>ACGTGGTGAGGAAAGGCAACCTTGC</td>
</tr>
</tbody>
</table>

### 2.1.4 Immunohistochemical Expression

Immunohistochemistry (IHC) for p53, p16, CD34 and PTEN was performed on all SFTs using the TMA, and, for PTEN, on full-section slides because of ambiguity of expression on the TMA. Paraffin sections at 4µm thickness were dried at 60°C oven before staining. The IHC was performed according to the manufacture’s guidelines using BenchMark XT-an automated slide strainer (Ventana Medical System). Antibodies for PTEN (Cell Signaling Technology, Rabbit monoclonal, #9559; 1:100), P16 (CINtec, mtm9517), P53 (Vantana, D07), and CD34 (Dako QBend10; 1:50) were used. For PTEN, biotinylated anti-rabbit IgG (Vector, BA-1000) was added to the slides, and the primary-secondary antibody complex was visualized with Ventana iView DAB Detection Kit. The rest of the antibodies were stained using Ventana Ultraview.
Universal DAB Detection Kit, which contains a cocktail of enzyme labeled secondary antibodies that locate the bound primary antibody. The complex is then visualized with hydrogen peroxide substrate and 3, 3'- diaminobenzidine tetrahydrochloride (DAB) chromogen, which produce a dark brown precipitate that is readily detected by light microscope. The slides were counterstained with Ventana Hematoxylin II and Bluing reagent, dehydrated in graded alcohol, cleared in xylene and coverslipped in Permount.

SFTs were scored for PTEN IHC as 2+ (positivity similar to normal stromal cells), 1+ (weakly positive), and 0 (negative). p53, p16 and CD34 were assessed according to their intensity (0, negative; 1, mild; 2, moderate; 3, strong). In addition, p16 and p53 staining was semi-quantitatively scored using the histology score (H-score) based on the intensity and the distribution of the positivity [H-score = 3 x (% of cells with 3+) + 2 x (% of cells with 2+) + 1 x (% of cells with 1+) with a maximum value of 300]. To exclude background non-specific staining, H-score more than 10 was considered positive.

2.1.5 Bioinformatics and biostatistics

2.1.5.1 OncoScan Copy Number Data

Analysis of the OncoScan MIP data was carried out using Nexus Copy Number™ v.6.0.beta (BioDiscovery, Howthorne CA). Samples with median absolute pair-wise difference (MAPD) value of more than 0.6 were excluded. Within each sample, probes that demonstrated a call rate of <90% and relative standard deviation of >30% were excluded from further analysis. Probes are then clustered using the SNP-FASST2 algorithm. The significance threshold that determined the clustering was set to 5.00E-07 according to the software provider’s previous experience with the 330K-probe OncoScan. The minimum number of probes required to create a segment was set to three, and the maximum spacing between adjacent probes before breaking a segment was set
to 1 mega base pairs. The median of the Log$_2$ ratio of each segment is calculated and used for copy number estimation. A segment with considered to have normal CN, CN gain, high gain, loss and big loss if its log ratio value was zero, >0.3, >1.2, <-0.3, and <-1.2, respectively. Any altered chromosomal region with at least 75% reported CNVs, and those present in the control non-tumour samples, were reported separately, and excluded from further analysis. Descriptive statistics were used to report and compare changes among SFTs, and among those that recurred, and those that contained pathological predictors of malignancy. Genomic instability was assessed through comparing the fragment genome altered for each subgroup using Mann-Whitney U test.

2.1.5.2 Fluorescence in situ Hybridization (FISH)

After signal enumeration, cells with less than two centromeric signals were excluded. For the valid cells, the gene:CEP signal ratio was calculated for each cell, and averaged for all cells per sample.

For $C\text{-}MYC$ gene copy gains/amplifications evaluation, the thresholds used were adopted from those previously described in another soft tissue tumour$^{138}$. Cells with no signals or with signals of only one colour were disregarded. Tumor cells displaying at least two centromeric chromosome 12 signals and multiple $C\text{-}MYC$ signals, with an average $C\text{-}MYC$:CEP12 ratio of $\geq 2$, were considered consistent with amplification/high copy number gains of the $C\text{-}MYC$ gene. Tumour cells displaying at least two centromeric chromosome 12 signals and an equal number of $C\text{-}MYC$ signals, with a $C\text{-}MYC$:CEP12 ratio of less than 2, were considered consistent with no amplification of the $C\text{-}MYC$ gene. The percentage of cells with 3 copies of $C\text{-}MYC$ gene (low copy gain), and those with 4 or more copies was calculated. As the control probe was not from chromosome 8, detection of trisomy or polysomy was not achievable.
Similarly, evaluation of PTEN and RB1 loss was performed after excluding cells with less than 2 signals of the CEP probe. Tumours with an average gene:control ratio of 0-0.5, with a minimum of 20% of cells with gene loss\textsuperscript{139} were considered to have hemizygous loss of this gene, while those with a ratio of 0 were regarded as consistent with homozygous copy number loss. The percentage of cells with one, or zero target gene signals was calculated for each tumour. As the centromeric probe in the PTEN FISH experiment belongs to the same chromosome (chromosome 10), the percentage of cells with PTEN loss is potentially underestimated in cases with chromosome 10 monosomy.
Chapter 3: Results

3

3.1 Study Population

3.1.1 Patients and Tissue Samples

Nineteen patients with pleural SFTs were initially identified, six of which had at least one local recurrence (Table 7 and table 9). Tumour and non-tumour FFPE blocks from all the 19 cases, and from 3 matching recurrences, were available. After DNA extraction, two cases, SFT #13T1 and #18T1, were eliminated from genomic analysis because of the low quality of the extracted genomic DNA (A260/A230 ratio for these cases was 1.39 and 1.15, respectively). DNA from all the remaining 17 SFTs, from 3 recurrences and from 11 non-tumour areas (5 matching and 6 non-matching cases) was submitted for OncoScan™ platform study. OncoScan data quality was satisfactory in all non-tumour samples, and in 19 of 20 tumour samples (16 primary and 3 matching recurrent SFTs), denoted by MAPD value of \( \leq 0.6 \); the only case with high MAPD value (#4T1) was excluded from further MIP data analysis.

When the TMA was being constructed, three newly-resected SFTs, including one recurrent tumour, were found and added to the original collection of the 19 SFT cases, raising the total number of samples on the TMA to 26 from 22 patients (20 primary; 2 recurrences from case #6, one recurrence from each of cases #8, 9 and 22, and only recurrence from #14). The male: female ratio in these 22 patients was 1.2:1, and median age at the time of diagnosis was 62 ± 14.1 years (range 37-85 years). The median follow-up duration was 3.43 ± 2.38 years (range 0.04-8.17 years), and the duration between the first resection and the discovery of a recurrence ranged from 2 – 6 years.
3.1.2 Pathologic Characteristics

Three tumour features were investigated: mitotic activity, necrosis and the maximum tumour dimension (Table 7 and Table 8). The median mitotic count was $1.5 \pm 5.8$ (range 0-22)/10 HPFs, with 12 SFTs from 9 patients exhibiting at least 4 mitoses/10 HPFs. Necrosis was recognized in 5 samples from 4 patients (3 primary and 2 recurrent); four of these 5 samples contained at least 4 mitoses /10 HPFs. The median tumour dimension was $7.7\pm7$ (range 1-26) cm. When excluding recurrences, seven of 21 (33.33%) SFTs have a maximum dimension of at least 10 cm; three, and two of these seven tumours demonstrated at least 4 mitoses/10HPFs, and necrosis, respectively. Thirteen of the 23 SFTs (56.5%) had any of the 3 pathologic features of malignancy, while only one SFT (#19T1) exhibited all the three features.

In order to investigate the relationship between these three pathologic features (necrosis, high mitotic activity and large tumour size) with tumour recurrence, we analyzed the presence of pathological features of malignancy among primary tumours that recurred (n=5) with those that did not (n=15) (Table 10). The proportion of SFTs with at least one of these 3 features was higher in SFTs that recurred than those that did not recur (5 of 5, 100% vs. 5 of 15, 33.3%; likelihood ratio, 8.63; 2-sided Fisher’s Exact test p=0.033). We then compared the rate of recurrence among tumours showing each one of these pathologic features (Table 10). SFTs with high mitotic count were more likely to recur than those without (4 of 6, 66.7% vs. 1 of 14, 7.1%; likelihood ratio, 5.079; 2-sided Fisher’s Exact test p=0.014). In contrast, the relationships between the presence of necrosis and recurrence (2 of 3, 66.7% vs. 3 of 17, 17.6%; likelihood ratio, 2.830; 2-sided Fisher’s Exact test p=0.140) and large tumour size and recurrence (3 of 7, 42.9% vs. 2 of 13, 15.4%; likelihood ratio, 1.770; 2-sided Fisher’s Exact test p=0.290) were not statistically significant, and were limited by the low number of events and low statistical power.
Table 7: Summary of clinicopathologic data on the pleural SFT study cases. Cases used for analysis by OncoScan MIP platform or for the TMA are identified.

<table>
<thead>
<tr>
<th>ID</th>
<th>Age at Diagnosis (years)</th>
<th>Sex</th>
<th>Rec.</th>
<th>Time to Rec. (years)</th>
<th>Maximum Dimension (cm)</th>
<th>Necrosis</th>
<th>Mitoses/10 HPF</th>
<th>OncoScan MAPD</th>
<th>TMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>64</td>
<td>F</td>
<td>no</td>
<td>5.5</td>
<td>absent</td>
<td>0</td>
<td>yes</td>
<td>0.38</td>
<td>yes</td>
</tr>
<tr>
<td>T1</td>
<td>52</td>
<td>M</td>
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<td>43</td>
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<td>present</td>
<td>1</td>
<td>yes</td>
<td>0.38</td>
<td>yes</td>
</tr>
<tr>
<td>T1</td>
<td>70</td>
<td>M</td>
<td>no</td>
<td>12.5</td>
<td>absent</td>
<td>4</td>
<td>no</td>
<td>NA</td>
<td>yes</td>
</tr>
<tr>
<td>T1</td>
<td>42</td>
<td>F</td>
<td>yes</td>
<td>4</td>
<td>present</td>
<td>4</td>
<td>yes</td>
<td>0.34</td>
<td>yes</td>
</tr>
<tr>
<td>T1</td>
<td>43</td>
<td>F</td>
<td>no</td>
<td>8.4</td>
<td>absent</td>
<td>1</td>
<td>no</td>
<td>NA</td>
<td>yes</td>
</tr>
<tr>
<td>T1</td>
<td>54</td>
<td>F</td>
<td>no</td>
<td>1</td>
<td>absent</td>
<td>1</td>
<td>no</td>
<td>NA</td>
<td>yes</td>
</tr>
<tr>
<td>T1</td>
<td>60</td>
<td>M</td>
<td>yes</td>
<td>6</td>
<td>25.5</td>
<td>absent</td>
<td>9</td>
<td>no</td>
<td>NA</td>
</tr>
</tbody>
</table>

* a recurrence five years after resection of primary tumour; ** a second recurrence three years after resection of first recurrence; § a recurrence five years after resection of primary tumour; # a recurrence two years after resection of primary tumour; §§ a recurrence six years after primary tumour resection, tissue from primary tumour is unavailable. HPF, high-power fields; MAPD, median absolute pair-wise difference; NA, not applicable; Rec., recurrence; TMA, tissue microarray.
Table 8: SFTs sorted according to their behavior and morphology

<table>
<thead>
<tr>
<th>ID</th>
<th>Pathologically malignant</th>
<th>No. of malignancy features</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>6T1</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
</tr>
<tr>
<td>6T2</td>
<td>yes</td>
<td>2</td>
<td>yes</td>
</tr>
<tr>
<td>6T3</td>
<td>yes</td>
<td>2</td>
<td>yes</td>
</tr>
<tr>
<td>7T1</td>
<td>yes</td>
<td>2</td>
<td>yes</td>
</tr>
<tr>
<td>8T1</td>
<td>yes</td>
<td>2</td>
<td>yes</td>
</tr>
<tr>
<td>8T2</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
</tr>
<tr>
<td>9T1</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
</tr>
<tr>
<td>9T2</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
</tr>
<tr>
<td>14T1</td>
<td>yes</td>
<td>1</td>
<td>yes</td>
</tr>
<tr>
<td>19T1</td>
<td>yes</td>
<td>3</td>
<td>yes</td>
</tr>
<tr>
<td>22T1</td>
<td>yes</td>
<td>2</td>
<td>yes</td>
</tr>
<tr>
<td>2T1</td>
<td>yes</td>
<td>1</td>
<td>no</td>
</tr>
<tr>
<td>15T1</td>
<td>yes</td>
<td>1</td>
<td>no</td>
</tr>
<tr>
<td>16T1</td>
<td>yes</td>
<td>1</td>
<td>no</td>
</tr>
<tr>
<td>17T1</td>
<td>yes</td>
<td>2</td>
<td>no</td>
</tr>
<tr>
<td>18T1</td>
<td>yes</td>
<td>2</td>
<td>no</td>
</tr>
<tr>
<td>1T1</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>3T1</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>4T1</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>5T1</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>10T1</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>11T1</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>12T1</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>13T1</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>20T1</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>21T1</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
</tbody>
</table>
**Table 9: Summary of CN events involving a major chromosomal segment.** SFTs are sorted according to the presence of copy number changes and clinicopathologic features of malignancy. (Blue bars, gains; red bars, losses)

| SFT | 6T3 | 9T1 | 9T2 | 8T1 | 8T2 | 10T1 | 10T2 | 11T1 | 11T2 | 12T1 | 12T2 | 13T1 | 13T2 | 14T1 | 14T2 | 15T1 | 15T2 | 16T1 | 16T2 | 17T1 | 17T2 | 18T1 | 18T2 | 19T1 | 19T2 | 20T1 | 20T2 | 21T1 | 21T2 | 22T1 | 22T2 |
|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| malignant | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes |

### Chromosome

| Chromosome | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| 1 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 2 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 3 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 4 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 5 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 6 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 7 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 8 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 9 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 10 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 11 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 12 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 13 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 14 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 15 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 16 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 17 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 18 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 19 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 20 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 21 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |
| 22 p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q | p q |

### Necrosis

| Necrosis | yes | yes | yes | no | yes | yes | no | yes | no | no | no | no | no | no | no | no | no | no | no | no | no | no | no | no |

### Mitoses

| Mitoses | yes | yes | yes | yes | yes | yes | yes | no | no | no | no | no | no | no | no | no | no | no | no | no | no | no | no | no |

### Size cm

| Size cm | 6.5 | 26 | 2.1 | 6.7 | 7.9 | 12 | 4.5 | 9 | 7.5 | 16 | 13 | 22 | 8 | 3.1 | 3.3 | 1.3 | 2.6 |

### Behaviour

| Behaviour | recurrence | primary | recurrence | primary | primary | recurrence | recurrence | non-recurrent | recurrence | non-recurrent | non-recurrent | non-recurrent | non-recurrent | non-recurrent | non-recurrent | non-recurrent | non-recurrent | non-recurrent | non-recurrent | non-recurrent | non-recurrent |
|-----------|------------|---------|------------|---------|---------|------------|------------|---------------|------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
### Table 10: Recurrence in pathologically malignant SFTs

<table>
<thead>
<tr>
<th>Feature</th>
<th>n Patients</th>
<th>n Samples</th>
<th>n Primary pSFTs</th>
<th>n Recurrence pSFTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitoses 4+/10HPF</td>
<td>9/22 (41%)</td>
<td>12/26 (46%)</td>
<td>5 (recurred 4/5 LR: 5.079 P=0.014)</td>
<td>7</td>
</tr>
<tr>
<td>Necrosis</td>
<td>4/22 (18%)</td>
<td>5/26 (19%)</td>
<td>3 (recurred 2/3)</td>
<td>2</td>
</tr>
<tr>
<td>Size 10+ cm</td>
<td>8/22 (36%)</td>
<td>8/26 (31%)</td>
<td>7 (recurred 3/7)</td>
<td>1</td>
</tr>
<tr>
<td>Any</td>
<td>12/22 (55%)</td>
<td>16/26 (62%)</td>
<td>12 (recurred 7/12)</td>
<td>4</td>
</tr>
</tbody>
</table>
3.2 Genomic Copy Number Events in Pleural Solitary Fibrous Tumours

3.2.1 Whole Genome Copy Number Analysis

As mentioned above, the total number of pleural SFTs with assessable OncoScan data was 19 (16 primary tumours and 3 matching recurrences). The copy number changes in all SFTs generated from this platform are summarized in Figure 7 and Table 9, and listed in details in appendices 1-2, and in Figure 8. Copy number variations were listed in appendix 3.

3.2.1.1 Copy Number Alterations in all SFTs Under Study

The fragment of genome altered (FGA) for all 19 SFTs ranged from 0.08% – 20.85% (Figure 6), and was significantly higher than that of non-tumour samples (median FGA, 0.32% ± 6.64% vs. 0.05% ± 0.02%, respectively; Mann-Whitney U, 10; 2-sided p value, <0.0001).

![Figure 6: Fragment of genome altered. SFTs are sorted according to copy number changes and pathological features as in Table 9](image-url)
There were more CN losses than gains (the length of genome affected with CN losses and CN gains for all SFTs was 1,136,044,066 bps and 731,786,071 bps, respectively). These alterations were detected in regions of varying sizes; the algorithm used to analyze the microarray generated 168 chromosomal segments with CN changes, ranging from 34 kilo base pairs (Figure 9) to major chromosomal regions or arms (partial aneuploidy) and entire chromosomes (aneuploidy).

![Figure 7: Frequency of copy number changes in all SFTs under study.](image)

Aneuploidy, in the form of gains (trisomy and polysomy) and losses (monosomy), were identified in 7 chromosomes from 5 SFTs (Table 11), while partial monosomy was detected in 5 different chromosomal arms in 5 SFTs (Table 12). Some of these events recurred in more than one SFT, involving regions of either comparable size (e.g. chromosome 8 gain and 13q loss), or of smaller chromosomal length (e.g. loss of chromosome 10 or of long arm of chromosome 10). These changes are discussed in more details under “Recurrent and Remarkable Copy Number Changes – an In-depth Examination” in page 65.
Table 11: Summary of SFTs with aneuploidy (Mbp, million base pairs)

<table>
<thead>
<tr>
<th>Chromosome Gain</th>
<th>Chromosome Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chromosome</strong></td>
<td><strong>SFT ID</strong></td>
</tr>
<tr>
<td>5</td>
<td>19T1</td>
</tr>
<tr>
<td>8</td>
<td>6T3, 19T1</td>
</tr>
<tr>
<td>16</td>
<td>19T1</td>
</tr>
<tr>
<td>19</td>
<td>19T1</td>
</tr>
<tr>
<td>20</td>
<td>19T1</td>
</tr>
</tbody>
</table>
Figure 8: The solitary fibrous tumours genome. Copy number gains (blue bars) and losses (red bars) in the somatic chromosomes are illustrated for all SFTs combined (A), and for each of the tumours individually (B).
Beside the CN events in major chromosomal structures, alterations affecting substantially large chromosomal segments of at least one million base pairs (Mbps) in length were identified in 36 of 168 segments with CN changes (Table 13; details of, and genes contained in these segments are listed in appendices 1-2). These alterations include low CN gains (21 segments in 3 SFTs), high CN gains (4 segments in 1 SFT), and hemizygous loss (11 segments in 8 SFTs). It is worth noting that the majority of the large-segment copy number alterations, as well as the aneuploidy mentioned above, occur in a limited number of SFTs, most notably in SFT# 19T1 and 8T2 (FGA, 20.85% and 20.45%, respectively; Figure 6), and that these alterations commonly recur among various SFTs.

The remaining changes, detected in smaller chromosomal segments, occurred across 19 somatic chromosomes, with the median length of affected segment being 372,511 ± 243,694.4 bps.

Table 12: Summary of SFTs with partial aneuploidy (Mbps, million base pairs)

<table>
<thead>
<tr>
<th>Chromosomal Region</th>
<th>Partial Monosomy</th>
<th>SFT ID</th>
<th>Region Length (Mbps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p</td>
<td></td>
<td>8T1</td>
<td>114</td>
</tr>
<tr>
<td>1p</td>
<td></td>
<td>8T2</td>
<td>117</td>
</tr>
<tr>
<td>9p</td>
<td></td>
<td>8T1</td>
<td>34</td>
</tr>
<tr>
<td>9p</td>
<td></td>
<td>8T2</td>
<td>35.5</td>
</tr>
<tr>
<td>10q</td>
<td></td>
<td>6T3</td>
<td>82</td>
</tr>
<tr>
<td>13q</td>
<td>6T3, 10T1, 19T1</td>
<td></td>
<td>88</td>
</tr>
<tr>
<td>15q</td>
<td></td>
<td>8T2</td>
<td>82</td>
</tr>
</tbody>
</table>
Table 13: Copy number abnormalities in large chromosomal segments (≥ one million base pairs). The range and median value of the length of the genomic segments are indicated.

<table>
<thead>
<tr>
<th>Chromosomal Region</th>
<th>SFT</th>
<th>Number of Segments with CN abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>2q</td>
<td>8T2</td>
<td>2</td>
</tr>
<tr>
<td>3q</td>
<td>8T2</td>
<td>1</td>
</tr>
<tr>
<td>5q</td>
<td>8T2</td>
<td>2</td>
</tr>
<tr>
<td>6p</td>
<td>8T2</td>
<td>1</td>
</tr>
<tr>
<td>6q</td>
<td>8T2</td>
<td>2</td>
</tr>
<tr>
<td>7p</td>
<td>8T1</td>
<td>1</td>
</tr>
<tr>
<td>7q</td>
<td>8T2</td>
<td>2</td>
</tr>
<tr>
<td>12p</td>
<td>16T1</td>
<td>1</td>
</tr>
<tr>
<td>12q</td>
<td>8T2</td>
<td>1</td>
</tr>
<tr>
<td>13q</td>
<td>8T2</td>
<td>2</td>
</tr>
<tr>
<td>14q</td>
<td>8T2</td>
<td>2</td>
</tr>
<tr>
<td>17q</td>
<td>8T2</td>
<td>3</td>
</tr>
<tr>
<td>22q</td>
<td>8T2</td>
<td>1</td>
</tr>
</tbody>
</table>

Total number of segments: 21

Segments Length
- Range: 1,037,308 - 13,224,233 bp
- Median: 1,432,412 ± 2,830,139 bp

<table>
<thead>
<tr>
<th>Chromosomal Region</th>
<th>SFT</th>
<th>Number of Segments with CN abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>13q</td>
<td>19T1</td>
<td>2</td>
</tr>
<tr>
<td>18p</td>
<td>19T1</td>
<td>1</td>
</tr>
<tr>
<td>18q</td>
<td>19T1</td>
<td>1</td>
</tr>
</tbody>
</table>

Total number of segments: 4

Segments Length
- Range: 1,272,272 to 5,054,435 bp
- Median: 2,577,700 ± 1,686,327 bp

3.2.1.2 Copy Number Alterations in Pleural SFTs Correlate with Pathological Phenotype

The pattern of copy number alterations of the pathologically malignant SFTs (with necrosis, mitotic activity of a minimum of 4/10 HPFs or tumour maximum dimension of at least 10 cm), (n=13), was compared to that of the pathologically benign tumours that lacked all of these features (n=6) (Figure 10 and Table 9).
Pathologically malignant SFTs demonstrated more CN abnormalities, and significantly higher FGA than the rest of the SFTs (median FGA, 0.43% ± 8.5% vs. 0.14% ± 1.08%, respectively; Mann-Whitney U, 20.00; 2-sided p, 0.041). Most of the recurring CN changes in small chromosomal segments, which were identified in malignant SFTs (in 1q21, 1q44, 2p11, 7p14.1, 7q24, 9p11.2, 14q11.2, and 15q11.1), represent previously reported CNVs. Other recurring alterations affected larger segments of 5 chromosomal regions (Table 14). Of these recurrent alterations, losses in the short arm of chromosome 1 (1p) and that of chromosome 9 (9p) were discovered in a pair of samples from one patient (case# 8): a primary SFT (8T1), and a recurrence (8T2) (Further details are provided under “Pair-Wise Comparison of Primary and Recurrent SFTs” on page 61). Additionally, major parts of 13q were lost in 2 pathologically malignant SFTs (6T3 and 19T1) and in one benign tumour (10T1).

Table 14: Regions with copy number alterations, recurrent in pathologically malignant SFTs

<table>
<thead>
<tr>
<th>Chromosomal Region</th>
<th>Event</th>
<th>Histologically Malignant SFT(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p</td>
<td>CN loss</td>
<td>8T1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8T2</td>
</tr>
<tr>
<td>Chromosome 8</td>
<td>CN gain</td>
<td>6T3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19T1</td>
</tr>
<tr>
<td>9p</td>
<td>CN loss</td>
<td>8T1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8T2</td>
</tr>
<tr>
<td>10q</td>
<td>CN loss</td>
<td>6T3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8T2</td>
</tr>
<tr>
<td>13q</td>
<td>CN loss</td>
<td>6T3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19T1</td>
</tr>
</tbody>
</table>

On the other hand, all of CN changes in pathologically benign SFTs (illustrated in Figure 10) were reported as CNVs, except for the loss of 13q (mentioned above) and loss in 5 segments on 20q in SFT# 10T1 (Figure 11).
Figure 9: Copy number changes in chromosome 12 of SFT# 6T3, showing the smallest fragment with a copy number change identified, on 12q13.3. The upper panel shows the entire chromosome, with the region of interest marked with a rectangle. The lower panel illustrates the distribution of log₂ ratio of each probe in that region. The median value of log difference (horizontal black line) demonstrates no CN change in the region shown except for the small region on 12q13.3 (chr12:55,758,007-55,792,166) which encloses a gene encoding for an olfactory receptor OR6C75 (blue horizontal lines, thresholds for low and high CN gains; red horizontal lines represent thresholds for hemi- and homozygous loss; black line, median log value of probes in a region; yellow horizontal line, moving average of probe value in a region; blue bars and highlight, area of CN gain).
Figure 10: Genomic copy number alterations in SFTs with pathologic predictors of malignancy (upper panel, SFTs with necrosis, significant mitotic activity, or maximum tumour dimension of at least 10 cm, n=13; lower panel, pathologically benign SFTs without these features, n=6; bars represent frequency of cases with CN changes at each genomic locus, as in figure 6; only changes in somatic chromosomes are shown)
Figure 11: Copy number profile of chromosome 20 of SFT# 10T1, showing non-CNV events in pathologically benign SFT. Log₂ ratio for each of the probes is plotted against their chromosomal location. (A) Five regions on 20q show CN loss, represent some of the few non-CNV events in pathologically benign SFTs. An area within one of these regions -marked by a rectangle, is magnified in (B). This area encompasses GNAS gene, which encodes the ubiquitously expressed Gαs subunit of heterotrimeric G-proteins. Losses in this gene do not have a clear clinical significance. However, activating mutations in this gene have been reported in pituitary adenomas and a number of inherited disorders. GNAS T393C polymorphism has been associated with worse survival in patients with a number of tumours, including melanoma and bladder, colorectal, renal cell carcinomas. (blue horizontal lines, thresholds for low and high CN gains; red horizontal lines represent thresholds for hemi- and homozygous loss; black line, median log value of probes in a region; yellow horizontal line, moving average of probe value in a region; red bars and highlight, area of CN loss)

3.2.1.3 Copy Number Alterations in Pleural SFTs Correlate with Behaviour and Tumour Recurrence

Next, we explored the relationship between copy number changes in non-recurrent SFTs (n=10) with those in tumours that eventually recurred (n=5) (Figure 12). For the recurrent cases, only primary tumour profile was analyzed, i.e., CN data from the recurrences were excluded from this comparison.

The combined CN abnormalities in SFTs that recurred were similar to those of pathologically malignant SFTs, except for the absence of 10q loss, as this loss was found in tumour samples from recurrences that were excluded from this correlative analysis (Table 14). Similarly, CN
changes in non-recurrent SFTs mirrored those of benign SFTs that lacked any of the pathological predictors of malignancy (Figure 10).

Among the 5 primary SFTs that recurred, the repeated CN changes identified (in 1q21, 1q44, 2p11, 7p14.1, 7q24, 9p11.2, 14q11.2, and 15q11.1) were formerly reported CNVs, and were also identified in the non-recurrent SFTs. Most of the non-CNV alterations in these recurrent SFTs accumulated in 2 cases: SFT# 8T1 and 19T1 (Figure 8). Alterations in SFT# 8T1 included 1p, 5p and 9p hemizygous loss, while those in 19T1 comprises chromosomes 5, 16, 19, and 20 gains, 13q hemizygous loss, 13q high CN gain, and chromosome 18 high copy number gains (Figure 8). Non-CNV changes in non-recurrent SFTs are limited to loss of 13q and in 5 segments on 20q in SFT# 10T1 as described above (Figure 11). Although #10T1 did not recur, the follow-up for the patient with this tumour was not optimal and was only 10 months because of patient relocation, whereas the minimum duration between primary resection and a recurrence in this and in other studies was 2 years.143

Although more CN events were identified in the recurrent SFTs, the median FGA was not significantly different between SFTs that recurred and non-recurrent SFTs (0.26% ± 8.96% vs. 0.24 ± 1.01%; Mann-Whitney U, 21.00; 2-tailed p, 0.624). FGA assessment is limited by the low number of events and the resultant low statistical power.
Figure 12: Genomic copy number alterations in recurrent and non recurrent SFTs (upper panel, alterations in recurrent SFTs, n=5; lower panel, non–recurrent SFTs, n=10; only primary SFTs are included; bars represent frequency of cases with CN changes at each genomic locus, as in figure 6; only changes in somatic chromosomes are shown)
3.2.1.4 Pair-Wise Comparison of Primary and Recurrent SFTs

We then compared the differences in genomic imbalances between each of the primary SFTs and their corresponding recurrences for the three available pairs of samples (Figure 13 and Table 9).

**Figure 13: Genomic copy number alterations in 3 pairs of primary and recurrent SFTs.** Tumours from three patients (#6, 8, and 9) are presented on the three panels, the upper half of each represents the changes in the primary tumours (6T1, 8T1 and 9T1), while the lower half represents the recurrences (6T3, 8T2, and 9T2). Red and Blue frequency bars represent copy number loss and copy number gains, respectively. Only changes in somatic chromosomes are shown.

Generally, more significant CN events were identified in cases# 8 (8T1 and 8T2) and 6 (6T1 and 6T3) than in case# 9 (9T1 and 9T2) (Table 15, and Figure 13), as changes in the latter were
mostly CNVs, except for the presence of hemizygous loss of a 260-Kbp region at 3q13.31 in the recurrence sample 9T2 (Figure 14).

CN alterations were discovered more in recurrent tumour samples than in the primary samples (Table 15, Figure 13). Primary tumours from cases# 6 and 9 (6T1 and 9T1) demonstrated changes confined to already-reported CNVs, which were retained in the subsequent recurrence tumour samples. For case#8, while the majority of CN changes in 8T1 were CNVs that reappeared in 8T2, gains and losses in small chromosomal segments, listed in Table 16, were discovered only in the primary tumour 8T1 and not in the recurrence, or in any other SFT. Detailed analysis of each of these regions is provided in appendices 1-2, and is not discussed here because they were not present in other SFTs, and may have limited relevance. In contrast, major chromosomal events, exemplified by 1p and 9p loss, were detected in both primary (8T1) and recurrent (8T2) tumours.

**Table 15: Comparison of fragment genome altered (FGA) between pairs of primary and recurrent SFTs**

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<th>Patient ID</th>
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<td>20.45%</td>
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<tr>
<td>9</td>
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<tr>
<td></td>
<td>9T2</td>
<td>0.08%</td>
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</table>
Table 16: Copy number changes found in the primary tumour 8T1 but not in its recurrence 8T2.

<table>
<thead>
<tr>
<th>Chromosomal location</th>
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<tr>
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<td>CN gain</td>
</tr>
<tr>
<td>3q13.2</td>
<td>CN gain</td>
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<tr>
<td>4q12</td>
<td>CN gain</td>
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<tr>
<td>4q24</td>
<td>CN loss</td>
</tr>
<tr>
<td>4q32.1</td>
<td>CN gain</td>
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<tr>
<td>5p15.32 - p15.2</td>
<td>CN loss</td>
</tr>
<tr>
<td>9q21.13</td>
<td>CN gain</td>
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<td>CN gain</td>
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<td>12p12.3</td>
<td>CN gain</td>
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<td>12q21.33</td>
<td>CN gain</td>
</tr>
<tr>
<td>19q13.12</td>
<td>CN gain</td>
</tr>
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</table>
Figure 14: Comparison of copy number profile of chromosome 3 for a primary (#9T1) and a recurrent (#9T2) sample from patient #9. Log$_2$ ratio for each of the probes is plotted against their chromosomal location. (A) The primary tumour, 9T1, demonstrated normal chromosome 3 copy number. (B) In the recurrent sample, 9T2, all the examined chromosome 3 segments showed normal copy number, except for a hemizygous loss on a 260-Kbp region on 3q13.31, marked by a rectangle. (C) Detailed examination of the area with CN loss in 9T2, revealing the absence of known genes except for the distal 20kbp of a 632-kbp gene, called limbic system-associated membrane protein (LSAMP), a candidate tumour suppressor gene, recently implicated in osteosarcomas.

Although most of the repeatedly identified alterations in primary SFTs were CNVs, changes that were acquired in more than one recurrent tumour samples were confined to the loss of the long arm of chromosome 10 (Figure 13, Figure 17 and Figure 18), which was identified in SFT# 6T3 and 8T2. Other genomic events in recurrent tumours were discovered only in one of the recurrent...
samples, and included chromosome 8 high copy gain and 13q loss (6T3), and chromosome 4 monosomy and 15q hemizygous loss (8T2).

3.2.1.5 Recurrent and Remarkable Copy Number Changes – an In-depth Examination

Regions with copy number alterations that adhered to the following criteria were selected for further detailed analysis, and confirmation with fluorescence in situ hybridization:

1. Present in more than one SFT

2. Identified either exclusively or more commonly in SFTs that recurred or in those that contained at least one of the pathological predictors of malignancy

3. Not confined to CNVs

Three genomic regions satisfied these criteria (Table 7 and Table 14): chromosome 8, 10q and 13q.

Chromosome 8 copy number gain was discovered in 2 SFT samples: 19T1 and 6T3 (Figure 15 and Figure 16). SFT# 19T1 is a primary tumour that recurred 4 years after resection, while 6T3 is a second recurrence of a SFT that was discovered 8 years following the primary tumour diagnosis, and 3 years after the first recurrence resection. Both samples presented with at least 1 pathologic features of malignancy.

Loss of a single copy of 10q chromosomal region is the only CN abnormality present in the recurrent SFTs 6T3 and 8T2 but not in their matching primaries (Figure 17 and Figure 18). Once again, both samples presented with at least 1 pathologic feature of malignancy.
Loss of 13q was identified in 2 malignant SFTs: 19T1 and 6T3 (Figure 19 and Figure 20). Additionally, it was found in 10T1 (Figure 21), which is a SFT that did not display any pathologic feature of malignancy, and did not recur. However, as mentioned above, the follow-up for the patient with this tumour was not optimal and was only 10 months because of patient relocation, whereas the minimum duration between the primary resection and a recurrence in our study and others’ was 2 years\textsuperscript{143}. It is worth noting that the valid OncoScan probes only target the long arm of chromosome 13, which is an acrocentric chromosome; therefore, as the copy number status of this chromosome’s short arm is unknown, whether a complete loss of this chromosome had happened is elusive.

The relevance of changes in these regions arises from their potential role in tumourigenesis; copy number gains can augment the function of oncogenes, while losses may result in loss or reduction of function of tumour suppressor genes. Both events can induce, promote or sustain tumour growth and survival. Oncogenes on chromosome 8, and tumour suppressor genes on 10q and 13q were retrieved from on-line databases\textsuperscript{107,108}, and are listed in Table 17-

Table

<table>
<thead>
<tr>
<th>Oncogenes</th>
<th>Cytoband</th>
<th># of SFTs</th>
</tr>
</thead>
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<td>Symbol</td>
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</tr>
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</tr>
<tr>
<td>FGFR1</td>
<td>8p12</td>
<td>2</td>
</tr>
<tr>
<td>PLAG1</td>
<td>8q12</td>
<td>2</td>
</tr>
<tr>
<td>RUNX1T1</td>
<td>8q22</td>
<td>2</td>
</tr>
<tr>
<td>C-MYC</td>
<td>8q24.21</td>
<td>2</td>
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</table>
MAFA  v-maf musculoaponeurotic fibrosarcoma oncogene family, protein A (avian)  
PTK2  PTK2 protein tyrosine kinase 2  

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Log2 Ratio</th>
</tr>
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<tbody>
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<td>MAFA</td>
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</tr>
<tr>
<td>PTK2</td>
<td>8q24.3</td>
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</tr>
</tbody>
</table>

Figure 15: Copy number profile of chromosome 8 of SFT# 6T3. Log2 ratio for each probe is plotted against their location on the entire chromosome (A), and on C-MYC gene area (B). The median log2 ratio (black line) lies above the threshold for high copy number gain. (blue horizontal lines, thresholds for low and high CN gains; red horizontal lines represent thresholds for hemi- and homozygous loss; black line, median log value of probes in a region; yellow horizontal line, moving average of probe value in a region; blue bars and highlight, area of CN gain)

Figure 16: Copy number profile of chromosome 8 of SFT# 19T1. Log2 ratio for each probe is plotted against their location on the entire chromosome (A), and on C-MYC gene area (B). The median log2 ratio (black line) lies above the threshold for low copy number gain. (blue horizontal lines, thresholds for low and high CN gains; red horizontal lines represent thresholds for hemi- and homozygous loss; black line, median log value of probes in a region; yellow horizontal line, moving average of probe value in a region; blue bars and highlight, area of CN gain)
Table 18: Known tumour suppressor genes on 10q, and the number of SFTs demonstrating losses in this region.

<table>
<thead>
<tr>
<th>Tumour Suppressor Genes</th>
<th>Cytoband</th>
<th># of SFTs</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Any</td>
</tr>
<tr>
<td>Symbol</td>
<td>Full Name</td>
<td>10p11.23</td>
</tr>
<tr>
<td>BMI1</td>
<td>BMI1 polycomb ring finger oncogene</td>
<td></td>
</tr>
<tr>
<td>KLF6</td>
<td>Kruppel-like factor 6</td>
<td>10p15</td>
</tr>
<tr>
<td>SIRT1</td>
<td>sirtuin 1</td>
<td>10q21.3</td>
</tr>
<tr>
<td>PTEN</td>
<td>phosphatase and tensin homolog</td>
<td>10q23.3</td>
</tr>
</tbody>
</table>

Figure 17: Copy number profile of chromosome 10 of SFT# 6T3. Log2 ratio for each probe is plotted against their location on the entire chromosome (A), and on PTEN gene area (B). For a major part of 10q, the median log2 ratio (black line) lies below the threshold for hemizygous copy number loss. (blue horizontal lines, thresholds for low and high CN gains; red horizontal lines represent thresholds for hemi- and homozygous loss; black line, median log value of probes in a region; yellow horizontal line, moving average of probe value in a region; red bars and highlight, area of CN loss)
Figure 18: Copy number profile of chromosome 10 of SFT# 8T2. Log$_2$ ratio for each probe is plotted against their location on the entire chromosome (A), and on PTEN gene area (B). The median log$_2$ ratio (black line) lies below the threshold for hemizygous copy number loss for a major part of the gene. (blue horizontal lines, thresholds for low and high CN gains; red horizontal lines represent thresholds for hemi- and homozygous loss; black line, median log value of probes in a region; yellow horizontal line, moving average of probe value in a region; red bars and highlight, area of CN loss)

Table 19: Known tumour suppressor genes on 13q, and the number of SFTs demonstrating losses in this region.

<table>
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<th>Tumour Suppressor Genes</th>
<th>Cytoband</th>
<th># of SFTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symbol</td>
<td>Full Name</td>
<td>Any</td>
</tr>
<tr>
<td>LATS2</td>
<td>large tumor suppressor, homolog 2 (Drosophila)</td>
<td>13q11-q12</td>
</tr>
<tr>
<td>BRCA2</td>
<td>breast cancer 2, early onset</td>
<td>13q12.3</td>
</tr>
<tr>
<td>STARD13</td>
<td>StAR-related lipid transfer (START) domain containing 13</td>
<td>13q12-q13</td>
</tr>
<tr>
<td>RB1</td>
<td>retinoblastoma 1</td>
<td>13q14.2</td>
</tr>
<tr>
<td>INTS6</td>
<td>integrator complex subunit 6</td>
<td>13q14.3</td>
</tr>
</tbody>
</table>
Figure 19: Copy number profile of chromosome 13 of SFT# 6T3. Log₂ ratio for each probe is plotted against their location on the entire chromosome (A), and on RB1 gene area (B). The median log₂ ratio (black line) falls below the threshold for hemizygous copy number loss for all 13q, and below that for homozygous loss for the region enclosing part of RB1 gene. (blue horizontal lines, thresholds for low and high CN gains; red horizontal lines represent thresholds for hemi- and homozygous loss; black line, median log value of probes in a region; yellow horizontal line, moving average of probe value in a region; red bars and highlight, area of CN loss)

Figure 20: Copy number profile of chromosome 13 of SFT# 19T1. Log₂ ratio for each probe is plotted against their location on the entire chromosome (A), and on RB1 gene area (B). The median log₂ ratio (black line) falls below the threshold for hemizygous copy number loss for a major component of 13q, including the regions RB1 gene. (blue horizontal lines, thresholds for low and high CN gains; red horizontal lines represent thresholds for hemi- and homozygous loss; black line, median log value of probes in a region; yellow horizontal line, moving average of probe value in a region; red bars and highlight, area of CN loss)
Figure 21: Copy number profile of chromosome 13 of SFT# 10T1. Log2 ratio for each probe is plotted against their location on the entire chromosome (A), and on RB1 gene area (B). The median log2 ratio (black line) falls below the threshold for hemizygous copy number loss for a major component of 13q, including the regions RB1 gene. (blue horizontal lines, thresholds for low and high CN gains; red horizontal lines represent thresholds for hemi- and homozygous loss; black line, median log value of probes in a region; yellow horizontal line, moving average of probe value in a region; red bars and highlight, area of CN loss)

3.2.2 Confirmatory FISH

Using the TMA, the copy number of the three selected genes was analyzed using fluorescence in situ hybridization.

3.2.2.1 C-MYC gene

In this experiment, the number of assessable samples was 19 of 26. The average C-MYC:CEP12 signal ratio ranged from 0.77-1.94 (median, 0.87 ± 0.3), confirming normal C-MYC gene copy number in the majority of the SFTs, except for 2 samples: 6T2 and 6T3 (Figure 22). As stated before, those 2 samples are recurrent tumours from the same patient.

SFT#6T2, a recurrence that was not analyzed by the OncoScan platform, had an average C-MYC:CEP12 ratio of 1.55, displaying the presence of at least one C-MYC gene copy gain. The percentage of tumour cells with 3 C-MYC copies was 15.4%, while that of cells with 4 or more copies (amplified) was 71.2%.
SFT#6T3 had the highest C-MYC:CEP12 ratio of 1.94, approaching the pre-set cut-off for amplification/high copy number gain of 2. Once again, the percentage of tumour cells with 3 C-MYC gene copies, and with 4 or more copies (amplified) was 14.3%, and 73.8%, respectively.

SFT#19T1 demonstrated on OncoScan panel the presence of low copy number gain of chromosome 8, including the region enclosing the C-MYC gene (Figure 16). On FISH, while the C-MYC:CEP12 signal ratio was 1.1, this tumour harboured the highest percentage of cells with a single C-MYC gene copy gain (SFT#19T1, 39.6%; median for all valid SFTs, 14% ± 9.2%). This might have been enough a gain to be detected by OncoScan but not by FISH. Another possibility to explain this inconsistency is tumour heterogeneity as different areas were selected for DNA extraction and for TMA construction.
Figure 22: C-MYC gene FISH results. The average C-MYC:CEP12 signal ratio (A), the percentage of cells with 3 C-MYC gene copies (B), and the percentage of cells with at least 4 C-MYC gene copies (C) are plotted for each SFT. Only tumours with at least 50 assessable tumour cells and those with nuclei containing at least 2 CEP12 signals are included.
3.2.2.2 PTEN gene

The number of valid samples was 24 of 26. The average PTEN:CEP10 signal ratio ranged from 0.50-1.02 (median, 0.97 ± 0.1) (Figure 23). The percentage of tumour cells with one PTEN copy was 0 – 84.4% (median, 6.7% ± 16.3%), and that of cells with homozygous PTEN loss was 0-6.3% (median, 0 ± 0.09%). SFT# 6T3 contained the highest percentage of cells with hemizygous (84.4%), and homozygous (6.25%) PTEN loss, with an average PTEN:CEP10 ratio of 0.50, confirming the loss of one PTEN copy relative to the centromeric control (Figure 17). The primary (#6T1) and the first recurrence (#6T2) on this case consistently demonstrated normal PTEN copy number (average PTEN:CEP10 ratio, 0.96 and 1.00, respectively). On the other hand, changes in SFT# 8T2 on OncoScan (Figure 18) were not replicated by the confirmatory FISH; the average PTEN:CEP10 ratio in this case was 0.90, and the percentage of cells with one and zero copies of PTEN was 15.9%, and 0.0%, respectively.
Figure 23: PTEN gene FISH results. The average PTEN:CEP10 signal ratio (A), the percentage of cells with hemizygous PTEN loss (B), and of cells with homozygous loss (C) are plotted for each SFT. Only tumours with at least 50 assessable tumour cells and those with nuclei containing at least 2 CEP10 signals are included.

Figure 24: PTEN FISH, showing normal CEP12 signal pattern (green) and loss of PTEN gene signals (red)
3.2.2.3 RB1 gene

The number of valid samples was 20 of 26. The average RB1:CEP12 signal ratio ranged from 0.14-0.98 (median, 0.90 ± 0.22) (Figure 25). The percentage of tumour cells with hemizygous RB1 loss ranged from 7.84%-62.65% (median, 17.2% ± 11.6%), and that of cells with homozygous loss was 0-78.4% (median, 1.3% ± 18.1%). Only 2 SFTs fulfilled the criteria of RB1 gene loss, displaying RB1:CEP12 ratio <0.5, and loss of 2 gene copies in > 20% of cells. These cases are 6T3 and 10T1.

SFT#6T3 demonstrated hemizygous loss of chromosome 13, and homozygous loss in the region of RB1 gene on OncoScan array (Figure 19). FISH confirmed these findings revealing a RB1:CEP12 average ratio of 0.17, and hemizygous and homozygous RB1 loss in 14.9% and 78.4% of tumour cells, respectively. In addition, it proved normal RB1 copy number in the primary and first recurrence of SFTs from this patient.

SFT# 10T1 also had hemizygous loss of chromosome 13 on the array (Figure 21), that was confirmed by FISH (RB1:CEP12 ratio, 0.33; percentage of cells with hemizygous and homozygous loss, 62.65%, and 31.33%, respectively).

Although SFT# 19T1 displayed low copy number loss on the OncoScan panel (Figure 20), its average RB1:CEP12 ratio was 0.71, and the percentage of tumour cells with hemizygous and homozygous RB1 loss was 25.97% and 3.90%, respectively. Despite the fact that these percentages do not meet the pre-set criteria for a copy number loss, this tumour has a lower RB1:CEP12 ratio and higher percentage of cells hemizygous for RB1 loss than the rest of SFTs with normal chromosome 13 copy number.
Figure 25: RB1 gene FISH results. The average RB1:CEP12 signal ratio (A), the percentage of cells with hemizygous RB1 loss (B), and of cells with homozygous loss (C) are plotted for each SFT. Only tumours with at least 50 assessable tumour cells and those with nuclei containing at least 2 CEP12 signals are included.

3.2.3 Selective Proteins Expression Analysis

We studied the expression of three proteins which were previously reported to carry a potential correlation with SFTs prognosis (Table 20). None of the SFTs expressed p53. In contrast, p16 was positive in 7 SFTs (27%), four of which (57%) are recurrent SFTs, and five (71%) demonstrated at least one of the pathological features of malignancy. CD34 was strongly and uniformly expressed in all SFTs, except for 3 cases (12%): 6T1 (weak positivity), 9T1 and 14T1 (complete negativity), all of which are either recurrent SFTs or SFTs that recurred after several
years of follow-up; for both 6T1 and 9T1, while the primary tumour did not express CD34, the recurrence re-expressed it. On the contrary, 14T1 is a recurrence sample.

PTEN immunohistochemical expression was evaluated on full histopathological sections of the 26 SFT samples. Six of the 26 SFT cases (23%) showed loss of PTEN expression either in the entire tumour, or in a region composing at least 50% of the tumour under study. Four of the six cases with PTEN complete or regional negativity (66.67%) represent a recurrent SFT or a primary tumour that recurred afterward (odds ratio, 3.71; 95% confidence interval, 0.54-25.59), and five of the six (83.3%) have at least one pathological feature of malignancy (odds ratio, 4.91; 95% confidence interval, 0.40-41.66). SFT# 6T3, which exhibited a hemizygous loss of PTEN, showed reduction in PTEN positivity (1+) in about 50% of the tumour cells, while the remaining half was completely negative (0). However, the primary tumour sample from this case (6T1) and the first recurrence (6T2) (both showing normal PTEN copy number) displayed a strong cytoplasmic (2+) and nuclear PTEN positivity (Figure 26). In contrast, both primary and recurrent tumours from patient #8, revealed negative (0) PTEN staining (Figure 27); SFT# 8T1 (with normal PTEN copy number) was weakly positivity (1+) in ~20% of tumour cells and negative (0) in the remaining cells, whereas 8T2 (hemizygous PTEN loss in 15.9% of cells) was completely negative (0) for PTEN. Furthermore, SFT# 22T1, which is a recurrent SFT sample, was negative for PTEN in more than 50% of cells. Two cases that did not recur also demonstrated at least regional PTEN immunohistochemical negativity; SFT# 17T1, a 16-cm tumour with necrosis, contained two copies of PTEN genes, and displayed PTEN IHC negativity in approximately 50% of tumour cells, whereas SFT#21T1, which had a maximum dimension of 13 cm, with no other feature of malignancy, had a normal PTEN copy number, but exhibited complete PTEN negativity.
<table>
<thead>
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Note: 0*: negative PTEN immunohistochemistry within more than 50% of tumour cells, but not the entire tumour is negative. §: cases with PTEN copy number loss
Figure 26: PTEN immunohistochemical expression in case#6. Representative microscopic fields of 6T1 (A), 6T2 (B), and 6T3 (C) is shown at 200X magnification.

Figure 27: PTEN immunohistochemical expression in case#8. Representative microscopic fields of 8T1 (A) and 8T2 (B) are shown at 200X magnification. (A) 8T1. (B) 8T2 (200X)
3.2.4 Mutations are Rarely Found in Pleural Solitary Fibrous Tumours

The number of eligible OncoScan mutation assays was 321 of the original 412 (77.9%) assays, from 46 genes. Using the service provider’s recommended thresholds, the number of assays highlighted for the potential presence of a mutation was 97 of 321 (30.2%), targeting 32 of 46 (69.6%) genes on 11 of 19 (57.9%) SFTs. Of these 11 SFTs with potential mutation, six (54.5%) contained a minimum of one of the pathologic feature of malignancy, and five of these six tumours are either a primary tumour that recurred, or the recurrence itself. The number of assays that were found to be potentially mutated on OncoScan in each of the SFTs is summarized in Figure 28, and a comparison between the number of assays per gene on OncoScan and the number of genes with potentially mutated assay is presented for all SFTs in Figure 29. Eleven potential mutations from 9 SFTs were selected to be confirmed by direct sequencing (Table 21). These mutations were selected in such a way so that they represented a range of cancer-related genes, being derived from the OncoScan mutation assays that were most frequently flagged among our study tumours. None of the sequenced samples showed any of the tested mutations. As these corresponded to the most commonly marked assays on OncoScan, further validation by sequencing was discontinued, and all possible mutations assays were considered to be not present in the SFTs.
Table 21: OncoScan mutation assays that were further assessed by Sanger sequencing

<table>
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<th>Potential mutations chosen for validation by Sanger sequencing</th>
<th>Number of SFTs</th>
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<td>APC_pE853X_c2557G_T</td>
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<tr>
<td>PTEN_pQ17X_c49C_T</td>
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<td>PTEN_pE235X_c703G_T</td>
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<td>NF2_pQ362X_c1084C_T</td>
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<td>EGFR_pD761Y_c2281G_T</td>
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</tr>
<tr>
<td>RB1_pR552X_c1654C_T</td>
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</table>
Figure 28: Panel chart, showing the number of OncoScan assays that satisfy the threshold for mutation across all valid SFTs.
Figure 29: Frequency chart of the number of OncoScan assays highlighted for potential mutations per gene, compared to the total number of assays for each gene.
Profiling the SFT cases with the custom mass spectrometry-based Sequenom panel revealed the presence of a mutation in paxillin in SFT# 15T1. This is a missense mutation resulting in substitution of alanine with threonine (p.A127T c.379G>A). Sequencing confirmed the presence of this mutation in the 59.5% of the cells in the tumour sample, and its absence from the matching non-tumour sample.

In addition, two forms of single nucleotide polymorphism (SNP) in exon 2 of the MET gene were detected in three SFTs, and were confirmed in their matching non-tumour tissue samples; one tumour (3T1) demonstrated a SNP at NC_000007.13:g.116339642G>T; E168D (rs55985569), and two SFTs (10T1 and 16T1) had a SNP at NC_000007.13: g.116340262A>G; N375S (rs33917957)\textsuperscript{147,148}. 
Chapter 4 : Discussion

4

SFTs are uncommon thoracic tumours, which can sometimes recur even after prompt resection, and can lead to the patients’ demise. Compared to other human neoplasms, they are under-studied, primarily because of their low frequency. The current study explores the genomic alterations in a mixed group of benign and malignant SFTs. Akin to the handful of studies on SFTs’ copy number and mutation profile, tumours in this study demonstrated low level of genomic instability, infrequent copy number alterations and rare mutations. Nevertheless, we show that these changes correlate to some extent to the tumours’ clinicopathological phenotype; higher levels of genomic instability, and more frequent copy number events were observed among SFTs with clinicopathological features of malignancy, including five primary SFTs that recurred and the four recurrent tumour samples (Table 9, Figure 10, Figure 12), providing evidence that clinicopathologic progression of SFTs entails a step-wise acquisition of genomic changes. Some of these changes involve genes of known role in cancer initiation and progression, such as 8q (including the oncogene c-myc) gain, 10q (including the tumour suppressor PTEN) loss, and 13q (including the tumour suppressor Rb1) loss. In this chapter, we review the genes altered in pSFTs, propose a potential model for SFT pathogenesis that connects the changes discovered in these genes, discuss the limitations of the current study and propose future directions for this project.

4.1 Genes Altered in SFTs

4.1.1 c-myc

c-myc is a proto-oncogene on 8q24. Its protein, c-Myc, is a transcriptional regulator, involved in both trans-activation and trans-repression through the recruitment of histone acetylases,
chromatin modulating proteins, basal transcriptional factors and DNA methyltransferase\textsuperscript{149}. It is estimated that c-Myc regulates the transcription of approximately 15\% of human genes including those implicated in cell cycle regulation (target genes include cyclins D1 and D2, \textit{CDK4}, and cyclin B1), protein synthesis (target genes include those encoding ribosomal RNAs and ribosome biogenesis proteins), cell adhesion and cytoskeleton related genes, and metabolism (for example, genes involved in glucose intake, glycolysis and iron metabolism)\textsuperscript{149}.

In cancer, \textit{c-myc} can be deregulated through mechanisms such as amplification, chromosomal translocation (such as between \textit{c-myc} and immunoglobulin heavy chain locus), and insertional mutations, therefore exerting its oncogenic effect, as mentioned above, through deregulating cell cycle control, enhancing cell growth (by promoting protein synthesis and metabolism), and inducing genomic instability and angiogenesis. Significant amplification of the 8q24 chromosomal region containing \textit{c-myc} and other genes was detected in approximately 41\% of tumours, particularly in breast (71\%), lung (56\%), ovarian (55\%), and colorectal (54\%) carcinomas\textsuperscript{150}.

In SFTs, the previous study showed gains in chromosome 8q in 2 of 12 (16.7\%) SFTs\textsuperscript{41}. In our study, 8q (and \textit{c-myc} gene) gains and amplification were detected in ~40-70\% of cells of the first and second recurrences of an SFT (#6), and the primary recurrent SFT (#19T1).

\subsection*{4.1.2 PTEN}
Phosphatase and tensin homologue (PTEN) gene is located at 10q23. It is a tumour suppressor gene that encodes a phosphatase that can act on both polypeptide and phosphoinositide substrates\textsuperscript{151}. Its tumour suppression activity mainly relies on opposing phosphoinositide 3-kinase (PI3K) function, leading to inactivation of AKT and mammalian target of rapamycin (mTOR) signaling. Loss of PTEN results in accumulation of phosphatidyl
inositol-3,4,5-trisphosphate (PtdIns(3,4,5)P3) and recruitment of the pleckstrin homology (PH) domain-containing proteins AKT and 3-phosphoinositide-dependent kinase 1 (PDK1). Activation of AKT drives cell survival, proliferation and cellular metabolism through phosphorylation of a number of proteins such as forkhead box O (FOXO) and p27. In addition, AKT induces inhibitory phosphorylation of tuberous sclerosis proteins and 40 kDa Pro-rich AKT1 substrate 1 (PRAS40), which are negative regulators of mTORC1, resulting in the activation of the latter, and consequent enhanced protein translation and cell survival\(^\text{151}\).

Furthermore, PTEN exerts a role in the double-stranded DNA repair and cell cycle control both through AKT-dependent action (e.g., by phosphorylation, ubiquitination, and reduced nuclear localization of Ser/Thr kinase checkpoint kinases 1, CHK1) or PI3K-AKT-mTOR pathway-independent (e.g., controlling chromosomal integrity by regulating RAD51-mediated DNA double strand break repair)\(^\text{152}\).

PTEN genetic and epigenetic alterations have been reported in a range of cancers. PTEN somatic mutations (reported in the “Sanger Institute Catalogue of Somatic Mutations in Cancer” web site, [http://www.sanger.ac.uk/cosmic](http://www.sanger.ac.uk/cosmic)\(^\text{133}\)) involve a variety of tumour subtypes, commonly endometrial carcinomas (37%), gliomas (17%), melanomas (14%), and prostate (14%), breast (6%) and colonic (6%) carcinomas. These mutations involve regions across almost the entire gene, with four most common mutations (Arg130Gly missense mutation, Arg173His missense mutation, Arg233 nonsense mutation, and Arg335 nonsense mutation). Additionally, PTEN gene deletions have also been reported in a number of tumours, such as prostate carcinoma\(^\text{153}\), breast carcinoma\(^\text{154}\), and melanomas\(^\text{155}\), and in soft tissue sarcomas, for example, leiomyosarcomas, in which this deletion was associated with aggressive behavior\(^\text{156-158}\). Moreover, loss of PTEN
tumour suppressor function can also ensue from methylation of cytosine-guanine (CpG) sites of its promoter in cancers including endometrial\textsuperscript{159}, gastric\textsuperscript{160} and non-small cell lung cancer\textsuperscript{161}.

The role of PTEN tumour suppression loss in promoting and enhancing tumourigenesis has been shown to be dose-sensitive, based on the degree of its inactivation. Both hemizygous and homozygous loss of PTEN are important in the initiation and progression of cancer, varying according to the tumour origin and differentiation\textsuperscript{162}. It has been proposed that in early stages of oncogenesis, induction and enhancement of oncogenesis by PTEN loss follows the “obligate haploinsufficiency model”, in which PTEN haploinsufficiency is more tumorigenic than when the gene is completely lost\textsuperscript{162,163}. A possible explanation of this model is that the homozygous loss of this gene (or another gene as a result of recombination during the ‘second hit’) in the early stages of cancer development would result in cell death or in senescence through activation of fail-safe senescence mechanism (e.g. wild-type p53)\textsuperscript{163}; therefore, cells with heterozygous loss would be selected for oncogenic transformation. On the other hand, complete loss of PTEN is restricted to more advanced carcinomas\textsuperscript{164}, and is related to loss of the senescence mechanisms\textsuperscript{163}.

In summary, PTEN is a recognized tumour suppressor, the loss of which has been revealed in several cancer types. This loss has recently been targeted by inhibiting the DNA repair enzyme poly(ADP-ribose) polymerase 1 (PARP1), and this new modality is being tested both in the laboratories and in the clinics, providing a new promising modality of targeted cancer therapy\textsuperscript{165,166}.

The early copy number study on SFTs reported loss of 10q in one of the twelve (8.3%) cases\textsuperscript{41}. In the current study, loss of one copy of PTEN was detected in the second recurrence of SFT#6 but not in the primary or the first recurrence. On the other hand, the MIP array study showed the
loss of one copy of PTEN in 8T2, which is the recurrence sample of SFT#8. However, FISH study demonstrated the hemizygous loss affecting ~15% of cells, a percentage of cells not high enough to fulfill the pre-set criteria defining hemizygous loss. This discrepancy is potentially the result of tumour heterogeneity (tumour areas selected for DNA extraction were different from those used for TMA construction), false positivity of the microarray study (array performance and/or bioinformatics), or low sensitivity of FISH. In any way, PTEN hemizygous loss seems to carry some pertinence to SFT pathogenesis.

At the protein expression level, cases with PTEN CN loss were at least partially negative for PTEN immunohistochemistry. However, PTEN regional or complete negativity was observed in four SFTs with normal gene copy number, implying the presence of other epigenetic mechanisms inactivating this gene. Although there was a tendency towards PTEN IHC negativity in pathologically malignant and/or recurrent SFTs, this was not statistically significant, and may be once again related to the limited number of study subjects and events.

4.1.3 Rb1

The retinoblastoma (*Rb1*) gene is a tumour suppressor gene, located at chromosome 13q14, the protein product of which regulates the cell cycle\textsuperscript{167}. RB protein inhibits the E2F group of transcription factors by binding to their transactivation domain and sequestering them from other transcriptional activators\textsuperscript{168}. In addition, it recruits chromatin-remodelling proteins such as histone deacetylases and histone methyl transferases, which bind to the promoters of E2F-responsive genes such as cyclin E, enhancing the stability of the histone-DNA complex, with resultant diminished accessibility of chromatin to transcription machinery\textsuperscript{167, 169, 170}. More recently, corruption of the RB pathway has been reported to promote chromosome segregation
errors, chromosome instability and aneuploidy through at least 3 proposed mechanisms: deregulation of mitotic proteins expression (such as spindle-assembly checkpoint MAD2, leading to chromosomal breaks and deletions), deregulation of replication progression (through altering nucleotide levels, inducing replication stress, fork stalling and double-strand DNA breaks), or deregulation of chromatin structure (through changing condensin II and cohesin distribution on the chromatin altering centromeric structure)\textsuperscript{171}.

\textit{Rb1} alterations were first discovered in patients with retinoblastoma\textsuperscript{172}. Biallelic germline inactivation of \textit{Rb1} results in hereditary retinoblastoma, while somatic inactivation would cause sporadic retinoblastoma\textsuperscript{173}. In addition, according to the “Sanger Institute Catalogue of Somatic Mutations in Cancer” web site, \url{http://www.sanger.ac.uk/cosmic} \textsuperscript{133}, \textit{Rb1} mutations have been observed in bladder carcinomas (29%), osteosarcoma (16%), ovarian carcinomas (11%), lung carcinomas (10%), gliomas (6%), and others. The most common mutations are substitutions and deletions\textsuperscript{133}; substitutions (nonsense, 28.6%; missense, 11.0%) affected almost the entire genomic sequence of \textit{Rb1}, with hotspots on Arg\textsuperscript{320}, Arg\textsuperscript{358}, Arg\textsuperscript{455}, Arg\textsuperscript{552}, and Arg\textsuperscript{579}. \textit{Rb1} deletions reported in this database were mostly frame-shift (17.6\% of mutations in \textit{Rb1})\textsuperscript{133}. Furthermore, chromosomal deletions at the 13q region (containing \textit{Rb1} and other genes) have been reported to have a potentially significant role in the initiation, growth and survival of several tumour types; multiple GISTIC analyses have revealed that deletion at this region was statistically significant in an overall of 30.85\% of cancers, including breast carcinomas (47.7\%), lung carcinomas (46.5\%), gliomas (46.3\%), prostate carcinomas (42.4\%), and hepatocellular carcinomas (32.2\%)\textsuperscript{150}. Additionally, imbalance in this region has been frequently discovered in soft tissue sarcomas, particularly malignant fibrous histiocytomas, leiomyosarcomas, osteosarcomas, and rhabdomyosarcomas \textsuperscript{157, 174-176}. 
In the previously reported study, four of the 12 cases (33.3%) showed losses on chromosome 13 in the region of \textit{Rb} \textsuperscript{41}. In our study, hemizygous and homozygous loss of 13q (and \textit{Rb} \textit{I} gene) were detected in \textasciitilde 28-78\% of tumour cells in two malignant and one benign SFT.

\subsection*{4.1.4 PXN}

Only one mutation was detected and validated in our population of SFTs. This is a missense mutation in paxillin (PXN) in SFT# 15T1. PXN, located at 12q24.23, encodes a 68kDa focal adhesion protein, which acts as a scaffold protein, interacting with structural and signalling proteins to regulate cell structure, adhesion, migration, and survival (Figure 30)\textsuperscript{177-179}. The carboxy-terminus of the protein controls its focal adhesion function; it contains four LIM domains, which are composed of two zinc finger domains, mediating protein-protein interaction and are present in proteins involved in cytoskeletal organization, organ development and oncogenesis\textsuperscript{180}. These domains represent the binding sites of multiple structural and regulatory proteins, such as tubulin and non-receptor tyrosine phosphatase, and their phosphorylation contributes to the regulation of focal adhesion targeting of PXN\textsuperscript{177}. The amino-terminus of PXN regulates its signalling activity; it harbours five LD motifs, which start with a leucine (L) and an aspartate (D) residues and are rich in leucin motifs\textsuperscript{181}. Each of these PXN LD motifs mediate multiple protein interactions that are both overlapping and specific\textsuperscript{177}. For example, LD1 directly interacts with integrin-linked kinase (ILK) and actopaxin, which play a role in stabilizing the interaction between plasma membrane and the actin cytoskeleton, in regulating cell survival, proliferation and tissue morphogenesis, and in controlling cell migration by modulating Rho GTPase signalling\textsuperscript{177}. PXN role in oncogenesis is being uncovered; PXN protein overexpression was observed in a number of tumours, including colon\textsuperscript{182}, esophageal\textsuperscript{183}, and breast\textsuperscript{184} carcinomas. In lung carcinomas, PXN expression was reported in \textasciitilde 48\%-70\% of tumours\textsuperscript{185-187}, being associated with poorer overall survival (hazard ratio, 2.62; 95\% CI, 1.58–4.37; \textit{P} < 0.001)
and relapse free survival (hazard ratio, 2.28; 95% CI, 1.37–3.77; P, 0.001) in patients with PXN-over-expressing tumours. This increased expression was either related to PXN gene amplification, as a response to reduction of miR-218 inhibitory effect, or was associated with PXN gene mutation. Jagadeeswaran et al. reported an overall PXN mutation rate of 9.4% in lung cancer, and only 2% in the remainder of the investigated tumours. They reported a total of 21 unique PXN gene mutations, affecting residues between LD1 and LD2 motifs, and on the LIM domains. The most commonly identified mutation was A127T substitution mutation affecting a residue between LD1 and LD2 PXN motifs. A127T mutation-carrying cells lines showed increased cell growth, colony formation and invasiveness both in vivo and in vitro, possibly by conferring resistance to calponin-mediated proteolysis of PXN protein.

The low frequency at which this mutation was detected in SFTs under study (1 of 19 tumours, 5.3%) makes its significance in SFT pathogenesis indeterminate.
Figure 30: Paxillin (PXN) protein structure and function. A variety of kinases are activated by adhesion and growth factors, resulting in the phosphorylation of PXN. Examples of known kinases and PXN phosphorylation sites are shown in the diagram. PXN activation by phosphorylation underlies this protein’s functions in controlling cellular adhesion and motility. The red asterisk marks the mutated amino acid residue. (PS: phosphoserine; PT: phosphothreonine; PY: phosphotyrosine)
4.2 Proposed SFT Pathogenesis – Suggestion of Stepwise Acquisition of Genomic Alterations

The prevalence of copy number alterations in the above mentioned genes in SFTs of different stages may suggest the sequence of events in which these alterations accumulated during tumour progression (Figure 31). For example, chromosome 13q losses (including the tumour suppressor \textit{Rb1}) were discovered in a benign, a primary malignant and a recurrence malignant SFTs. One can assume that this “benign” tumour is either a truly benign case that did not affect the patient’s survival after resection, or a tumour that would have recurred if the patient had been followed up for a longer period of time (duration of follow-up was 10 months due to relocation of patient, less than the minimum period to develop a recurrence - 2 years\textsuperscript{143}). As a result, it is possible to imply that 13q loss happens at an early stage of tumour progression. On the other hand, chromosome 8q gains (including the oncogene \textit{c-MYC}) were identified in a primary malignant and a recurrence malignant SFTs, but not in any benign tumour, therefore, it is possible that these changes occurred at a later stage. In contrast, chromosome 10q losses (including the tumour suppressor \textit{PTEN}) were confined to recurrent malignant SFTs, and were not significantly present in their matching benign counterpart, suggesting an even further and later acquisition of these changes. Because SFTs grow slowly, detection of these tumours may occur at a later stage, at which malignant progression has already started. Therefore, analyzing SFTs upon diagnosis for the presence of copy number changes in \textit{PTEN}, \textit{c-myc} and \textit{Rb1} may be of a potential prognostic benefit, and may alter the frequency of follow-up of patients carrying these somatic changes. However, this inference has a number of important limitations. Firstly, this study is limited by low number of subjects and infrequent copy number events. This limitation includes the lack of primary-recurrent tumour pairs for the recurrent tumours under study. Secondly, these
changes did not occur uniformly in SFTs with more or less a similar clinical course or pathological features. Once again, validation of the results on a larger cohort of patients is essential, to confirm the significance of these findings.

Furthermore, CN changes in 8q, 10q and 13q that were present more often among malignant SFTs were not mutually exclusive, and thus cannot be used to stratify SFTs. Other changes, such as copy number losses on 20q in one case (SFT#10T1, Figure 11), PXN A127T mutation in 15T1, and MET gene polymorphism are rare and no conclusion can be made at this stage.

Figure 31: A proposed model of pleural solitary fibrous tumours pathogenesis. See text for details

4.3 Limitations of this Study

This study is limited by a number of factors. Firstly, the low number of tumours analyzed, because of the relative rarity of pleural solitary fibrous tumours, has a potentially detrimental effect on the power of the findings and the inferences drawn from them in this study. In addition, only tissue of lower quality (FFPE) was available for these tumours, which limited the choices for genome-wide analysis methods, and affected the data quality. In addition, the limited number of patients prevented a powerful analysis of survival and correlation of survival with genomic
alteration. Furthermore, limitations of each of the methods used in this study have been discussed above (See: Experimental Design, Materials and Methods). Specifically, copy number and mutation analyses in this study were performed through array-based methods that involve limited coverage of the genome relative to the length of the human genome. This incomplete coverage is more significant in the case of mutation analysis, in which less assays covering a narrow range of genes were included. Finally, inherent to the initial aims and design of this study, there was no analysis of the balanced genomic alterations in SFTs, which can affect protein structure and expression level.

4.4 Future directions

While three genes (c-myc, PTEN and Rb1) were identified to potentially have a role in SFT progression, the biological relevance of these genes should be validated in vitro using SFT cell lines, by studying the effect of introducing or reversing the copy number changes in these genes on the tumour cell growth and migration. These cell lines can be newly developed from prospectively collected specimens of SFTs, or they can be from the previously reported SFT cell lines (such as NCI2004_11_17:C6894 malignant SFT cell line). This will be followed by in vivo experiments by transplanting the SFT cell lines into a xenograft model (such as murine), and analyzing the effect of si-RNA knockdown or gene reintroduction, on tumour xenograft formation, growth and metastasis development. We have already successfully transplanted a malignant SFT (ID# 8T2 in this study) into mice models, and tissue from this model can be revitalized and used for the functional experimentation. Expanding the in vivo model would allow detailed study of the effect of various targeted therapeutic and chemotherapeutic agents on SFTs harbouring varying genetic changes.
Because SFTs revealed low rate of copy number events and low level of genomic instability, and due to the fact that mutation analysis was comparatively limited, it is fair to assume that mutations play a substantial role in SFT initiation and promotion. Studying mutations in rare tumours has historically led to the discovery of genes that have a fundamental role in the progression of not only these rare tumours but also that of more common ones, such as studying retinoblastoma that led to the discovery of Rb1 gene. Because mutation analysis was rather imperfect in this study, we aim in the future to expand mutation screening by performing transcriptome sequencing of the SFTs; using massively parallel next-generation sequencing methods, we aim to characterize various RNA species within these tumours, and thus allowing the quantification of the transcription of various genes, and the identification of mutations (single nucleotide variation or translocations). Combining the results from transcriptome sequencing with the copy number data provided here, and with the functional analysis will allow the discovery of alterations driving the development and progression of SFTs and initiate a systematic discovery of drugs to target them.

4.5 Conclusion

Pleural solitary fibrous tumours are uncommon tumours that generally are stable genomically. However, as they progress and recur, they tend to acquire genomic copy number alterations, specifically chromosome 13q (including Rb1 tumour suppressor gene) copy loss, chromosome 8q (including c-myc oncogene) copy gains, and chromosome 10q (including PTEN tumour suppressor gene) loss. In this limited study, mutations were rare events, and did not involve known targetable driver mutations. Further detailed mutation and functional analyses are required to uncover mutations that SFTs depend on for their survival and progression.
References


Oxford University Press (distributor): Lyon


111. Hart A. Mann-Whitney test is not just a test of medians: differences in spread can be important. *Bmj* 2001; 323(7309):391-3.


Appendices

Appendix 1: Copy number alterations (CNAs) in SFTs, excluding copy number variations (CNV), sorted according to their chromosomal location. Examples of oncogenes and tumour suppressor genes, and the length of each segment in base pairs were indicated.

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4q11 - q35.2  CN Loss  8T2  

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**Gene List:**
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- VEGFC
- WWC2
- YTHDC1
- ZFP42
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- IGFBP7
- 8T1
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- CTNN2D2
- 8T1
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- 8T1
- 5q11.1 - q11.2 CN Gain
- 8T1
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- 8T2
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28,234,822

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1,624,896

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8q11.1 - q22.1 CN Gain

19T1

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8q11.1 - q24.3 High CN gain

6T3

BAALC, BOP1, COX6C, CTHRC1, CYP7A1, CYP7B1, EBAG9, ENPP2, EXT1, GGH, HAS2, MAFA, MYBL1, C-MYC, NBN, NDRG1, PLAG1, PSCA, PTK2, RECQL4, RNF139, RUNX1T1, SNAI2, TNFSF11B, TP53INP1, TP5D2, WWPI, ABR, ADCK5, ADCY8, ADHFE1, ANGPT1, ANXA13, AR, ARFGEF1, ASAP1, ASPH, ATAD2, ATP6V1C1, AZIN1, BAI1, Cox55, CA2, CA8, CCNE2, CDH17, CEBPD, CHCHD7, CHLS1, CNGB3, COL14A1, COMMD5, COP5S, CPA6, CPNE3, CRH, CSMD3, CYC1, DCAF13, DEPTOR, DERL1, DNAJC5B, DPYS, E2F5, EEF1D, EIF2C2, EIF3E, EIF3H, ESRP1, EYA1, FABP4, FABP5, FAM83A, FAM84B, FAM92A1, FBXO32, FBXO43, FOXH1, FSIP1, EZD6, GDAPI, GDF6, GEM, GI4, GML, GAPA1, GPT, GRHL2, GRNA, GSDMC, GSDMD, HEY1, IHLA1, HRSP12, HSFI1, IL7, IMPA1, KCNK9, KCNQ3, KHDRBS3, KIAA0196, KLF10, LAPTM4B, LRPI2, LRRC14, LY6E, LY6K, LY96, LYN, LYNX1, MAL2, MAPK15, MATN2, MCM4, MIR124-2, MIR30B, MIR30D, MIR661, MMP16, MOS, MHC, MTBP, MTDH, MTSS1, NAPRT1, NCOA2, NOV, NBWR1, NRBP2, NSMAF, NSMCE2, NUDC1, OC90, OPRK1, PABPC1, PAG1, PDP1, PENK, PLEC, POLR2K, POP1, POU5F1B, PPP1R16A, 99,298,419
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9p21.3 - p21.1 CN Loss 8T1 CDKN2B, TEK, CDKN2B-AS1, ELAVL2, IFNK, MTAP, PLAA, TUSC1 6,955,590

9p24.1 - p21.3 CN Loss 8T1 KDM4C, MLLT3, PSIP1, PTPRD, SH3GL2, ACER2, ADAMTS11, BNC2, DENND4C, IFNA1, IFNA13, IFNA17, IFNA2, IFNB1, MIR31, MIR491, MTAP, NFIB, PLN2, RPS6, TYRP1 14,977,833

9p24.3 - p21.3 CN Loss 8T2 JAK2, KDM4C, MLLT3, PSIP1, PTPRD, RLN2, SH3GL2, ACER2, ADAMTS11, AK3, BNC2, CD274, DENND4C, DMRT1, DMRT3, DOCK8, FOXD4, GLDC, IFNA1, IFNA13, IFNA17, IFNA2, IFNB1, IL33, INSL4, KANK1, MIR101-2, MIR31, MIR491, MLANA, MTAP, NFIB, PDCD1LG2, PLN2, RLN1, RPS6, SLC1A1, SMARCA2, TPD52L3, TYRP1, UHRF2, VLDLR 21,833,441

9p24.3 - p24.1 CN Loss 8T1 DMRT1, DMRT2, DMRT3, DOCK8, FOXD4, GLDC, IL33, INSL4, INSL6, JAK2, KANK1, MLANA, PDCD1LG2, PPAPDC2, RLN1, RLN2, SLC1A1, SMARCA2, TPD52L3, UHRF2, VLDLR 6,724,369

9q21.13 CN Gain 8T1 ALDH1A1, ANXA1 289,836

9q22.2 CN Loss 17T1 SYK 399,046

10p15.3 - p11.1 CN Loss 8T2 ABI1, AKR1C3, BMI1, GATA3, KLFL6, MLLTI0, MRC1, NET1, AKR1C1, AKR1C2, AKR1E2, ANKR3D0A, APBB1IP, ARHGAP12, ARHGAP21, ARMC3, BAMB1, CAMK1D, CCDC7, CCNY, CNFN, CELF2, CREM, CUL2, DCLRE1C, DNAJC1, EPC1, FAM188A, FBXO18, FRMD4A, FZD8, GAD2, GD2, GTPBP4, HSPA14, IL15RA, IL2RA, ITGA8, ITGB1, ITIH2, ITIHI5, KIF5B, KIN, MAP3K8, MASTL, MCM10, MSRB2, MYO3A, NMT2, NRP1, OLAH, OPTN, PAR3D, PFKFB3, PKP, PIP4K2A, PLXDC2, PRKCG, PRTFDC1, PTPLA, RAB18, RBM17, RSU1, SEPHS1, SUV39H2, TRD5MT1, UCN3, VIM, ZEB1 39,194,227

10q11.1 - q11.21 CN Loss 8T2 ALOX5, RET, CXCL12, HNRNPA3P1, HNRNPF, RASGEF1A, RASSF4 3,861,864

10q11.22 - q26.3 CN Loss 8T2 ADAM12, ADD3, AIFM2, BAG3, BNIP3, BTRC, CCDC6, DKK1, DMBT1, EIF3A, ERLC6, FGF8, FGF19, HTRA1, LDB1, LGI1, LOXL4, MAPK8, MXI1, NCOA4, NFKB2, PAX2 88,310,047
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10q11.23  |  CN Loss  |  6T3  |  ERCC6, CHAT, PGBD3  |  202,124

10q11.23 - q26.3  |  CN Loss  |  6T3  |  ADAM12, ADD3, AIFM2, BAG3, BNIP3, BTRC, CCDC6, DKK1, DMBT1, EIF3A, FGF8, FGF2, HTA1, LDB1, LGG1, LOX4, MXI1, NFKB2, PAX2, PDCD4, PSAP, PTEN, RHOBTB1, SIRT1, SNCG, ABCC2, ABLIM1, ACSL5, ACTA2, ACTR1A, ADAM8, ADAMTS14, ADK, ADRB1, AFAP1L2, ANK3, ANKRD1, ANKRD2, ANXA11, ANXA7, ARHGAP19, ARID5B, ARL3, AS3MT, ASCC1, BCCIP, BLNK, BLOC1S2, BMPR1A, BTAF1, BUB3, C10orf116, C10orf118, C10orf54, CAMK2G, CASP7, CCAR1, CDK1, CEP55, CHUK, COL17A1, COX15, CSTF2T, CTBP2, CTNNA3, CUE2D2, CUZD1, CYPI7A1, CYP26A1, CYP2C18, CYP2C19, CYP2C8, CYP2C9, CYP2E1, DCLRE1A, DDX4, DDX21, DHX32, 82,557,865
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- **16p12.1 CN Gain (19T1):** ABCC1, CIITA, CREBBP, DNAJA3, ERCC4, MYH11, PKD1, SOCS1, TRAP1, TSC2, USP7, ABCA3, ABCC6, ACSM1, ARL6P1, ATP6V0C, BACR4, BFA1, C16orf5, C16orf62, CCNF, CLDN6, CLDN9, CLUAP1, CRYM, DEXI, DNASE1, E4F1, EEF2K, EMP2, GDE1, GFER, GP2, GRN2A, GSP51, HAGH, HMOX2, IL32, LITAF, MEFV, MIR193B, MKL2, MMP25, NDE1, NTHL1, PAM16, PDK1, PGP, PKMYT1, PLA2G10, PPL, PRM2, PRSS21, PRSS41, RAB26, RPS2, RRN3, SLC9A3R2, SLX4, SMG1, TCEB2, TFAP4, TFRSF12A, TFRSF17, TRAF7, ZG16B
- **16p13.3 CN Gain (19T1):** ABCC1, CIITA, CREBBP, DNAJA3, ERCC4, MYH11, PKD1, SOCS1, TRAP1, TSC2, USP7, ABCA3, ABCC6, ACSM1, ARL6P1, ATP6V0C, BACR4, BFA1, C16orf5, C16orf62, CCNF, CLDN6, CLDN9, CLUAP1, CRYM, DEXI, DNASE1, E4F1, EEF2K, EMP2, GDE1, GFER, GP2, GRN2A, GSP51, HAGH, HMOX2, IL32, LITAF, MEFV, MIR193B, MKL2, MMP25, NDE1, NTHL1, PAM16, PDK1, PGP, PKMYT1, PLA2G10, PPL, PRM2, PRSS21, PRSS41, RAB26, RPS2, RRN3, SLC9A3R2, SLX4, SMG1, TCEB2, TFAP4, TFRSF12A, TFRSF17, TRAF7, ZG16B
- **16q11.2 CN Gain (19T1, 8T2):** ABC11, ADCY7, BRD7, DNAJA2, MYLK3, NDK1, NOD2, ORC6, SIAH1, VPS35
- **16q12.1 CN Gain (19T1):** AMFR, CYLD, MMP2, RBL2, AKTIP, ARL2BP, CCL17, CCL22, CDH8, CES1, CIAPIN1, CSNK2A2, CX3CL1, DOK4, FTO, GINS3, GNAO1, GPR56, IRX5, KIFC3, LOC643714, MIR138-2, MMP15, MT1A, MT1E, MT1F, MT1G, MT1H, MT2A, MT3, NDRG4, NOD2, NUP93, POLR2C, SLC12A3, SLC6A2, TOX3
- **16q21 CN Gain (19T1):** BCA1, CBFB, CDH1, CTCF, NOL3, NQO1, PHLP2, ZFHX3, ACD, CALB2, CDH11, CDH16, CDH3, CDH5, CES2, CFDP1, CHST4, CHTF8, CYB5B, DDX19A, DDX19B, DDX28, DHODH, DHX38, E2F4, FA2H, FHOD1, FUK, GLG1, HAS3, HP, HSD11B2, HYDIN, LCAT, MARVELD3, MIR328, MLKL, MTSS1L, NAE1, NFA5, NFA5C3, NOB1, PAR6A, PDF, PSKH1, PSMB8, PSMD7, RFWD3, RRAD, SF3B3, SLC12A4, SLC7A6, SMPD3, ST3GAL2, TAT, TERRF2, TERRFIP, TK2, TPP3, TRADD, VAC14, WWP2, ZNF23
- **16q24.3 CN Gain (8T2):** p53, SHBG, TP53, WRAP53, ATP1B2, CD68, CHRNA1, EFN3, EIF4A1, FGF11, FXR2, KCTD11, POLR2A, SAT2
- **17p13.1 CN Loss (6T3):** p53, SHBG, TP53, WRAP53, ATP1B2, CD68, CHRNA1, EFN3, EIF4A1, FGF11, FXR2, KCTD11, POLR2A, SAT2
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<td>EPB41L3, GATA6, RBBP8, ANKRD30B, APCDD1, CABLES1, CIDEA, CTAGE1, ESCO1, GNAL, GREB1L, IMPA2, L3MBTL4, LAMA1, MC2R, MIB1, MIR1-2, MIR133A1, PPP4R1, PGM2, PTEN, RAB12, RAB31, RALBP1, RIOL, ROCK1, TUBB6</td>
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Total CN Gain: 1,933,527
Total CN Loss: 464,988
Total High CN gain: 1,282,709
Total High CN loss: 1,272,272
Total Gene Symbols: 14,287,628
Total Gene Symbols: 163,566
Total Gene Symbols: 1,060,976
Total Gene Symbols: 336,498
Total Gene Symbols: 14,415,926
Total Gene Symbols: 557,780
Total Gene Symbols: 3,297,326
Total Gene Symbols: 18,329,050
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<td>19q13.33 - q13.43</td>
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**Probesets and Gene Counts:**
- 19q12 CN Gain: 2,893,948
- 19q12 - q13.12 CN Gain: 6,131,018
- 19q13.12 - q13.32 CN Gain: 10,661,818
- 19q13.32 CN Gain: 412,165
- 19q13.33 - q13.43 CN Gain: 9,118,951
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Appendix 2: Copy number alterations (CNAs) in SFTs, excluding copy number variations (CNV), sorted according to chromosomal event. Examples of oncogenes and tumour suppressor genes, and size of each segment in base pairs, are indicated.

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<th>Length</th>
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- 6p21.2: 1,236,969
- 6q23.2: 1,384,602
- 6q23.3: 1,419,908
- 7p15.1: 449,873
- 7p22.1: 265,156
- 7p22.3: 1,129,210
- 7q21.3: 599,919
- 7q31.1: 1,220,309
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9q21.13  CN Gain  8T1  ALDH1A1, ANXA1  289,836

11q13.4  CN Gain  16T1  69,890

11q22.3  CN Gain  8T1  PDGFβ  126,761

11q24.2  CN Gain  8T1  170,582

12p11.1 - q11  CN Gain  16T1  1,432,412

12p12.3  CN Gain  8T1  PIKC2G  228,833

12q13.11 - q13.13  CN Gain  8T2  ADCY6, ARF3, CCNT1, DDX23, DHK, DNAJC22, LALBA, LOC100335030, MCRS1, MLL2, PRKAG1, PRPH, RHEBL1, RND1, TMBIM6, TUBA1A, TUBA1B, WNT1  1,316,607

12q13.3  High CN gain  6T3  STAT6  34,160

12q21.33  CN Gain  8T1  DCN, LUM  383,642

12q23.1  CN Gain  8T2  ANKS1B, NR1H4, SCYL2  784,162

12q23.2  CN Gain  8T2  DRAM1, CHPT1, SYCP3  430,472

12q24.31 - q24.32  CN Gain  17T1  172,053

13q13.1  CN Gain  8T2  KL, PDS5B  886,838

13q13.2 - q13.3  CN Gain  8T2  STAR13, DCLK1, NBEA, RFC3  2,724,241

13q13.3 - CN Gain  8T2  FOXO1, LHFP  1,709,313
q14.11

13q14.11 CN Gain 8T2 DNAJC15, EPSTI1, TNFSF11 567,993

13q33.1 CN Gain 10T1 371,574

13q33.1 - q33.2 High CN gain 19T1 725,833

13q33.2 - q33.3 High CN gain 19T1 ARGLU1, EFNB2 1,858,074

13q33.3 - q34 High CN gain 19T1 ANKRD10, ARHGEF7, COL4A1, COL4A2, ING1, IRS2, LIG4, RAB20, SOX1, TNFSF13B 5,054,435

14q12 - q21.1 CN Gain 8T2 FOXA1, NKX2-1, PAX9, PRKD1, AKAP6, ARHGAP5, BAZ1A, BRMS1L, CFL2, COCH, EGLN3, FOXY1, MIPOL1, NFkBA, NKX2-8, PSMA6, SNX6, SRP54, STRN3 9,169,640

14q22.1 CN Gain 8T2 BMP4, CDKN3, CGRRF1 334,802

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14q23.1 CN Gain 8T2 RTN1 305,742

14q24.2 CN Gain 8T2 365,015

14q31.3 CN Gain 8T2 1,466,640

14q32.2 - q32.31 CN Gain 7T1, 16T1 MIR127, MIR376C, MIR380, MIR433, MIR493, MIR494, MIR495 231,823

14q32.31 CN Gain 8T2, 10T1, 15T1T1, 17T1 148,546

15q14 CN Gain 8T1 372,018

16p12.1 - p11.1 CN Gain 19T1 EIF3C, FUS, IL21R, MAPK3, MVP, PLK1, ALDOA, AQP8, ARHGAP17, ATP2A1, BCKDK, BCL7C, CD19, CDIPT, CHP2, CORO1A, COX6A2, CTF1, ERN2, HS3ST2, IL27, IL4R, INO80E, ITGAD, ITGAL, ITGAM, ITGAX, KIF22, LAT, MAZ, NMFCE1, NUPR1, ORAI3, PALB2, PHKG2, PPP4C, PRKCB, PRSS8, PYCARD, 12,466,572
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Copy number gains (CN gain) refer to an increase in the number of copies of a gene or region of DNA, while copy number losses (CN loss) refer to a decrease in the number of copies. The type of CN gain or loss is indicated by the notation (e.g., 19T1 for tandem duplication). The numbers following the gene list indicate the number of copies or significant changes. For example, 1,272,272 indicates the copy number, and 3,297,326 indicates the number of significant changes associated with that gene list.
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| 19q13.32 | CN Gain | 8T2 | HIF3A, IGFL1, PPP5C | 412,165 |
| 19q13.33 - q13.43 | CN Gain | 19T1 | ATF5, BCL2L12, KLK10, KLK11, KLK4, KLK5, KLK7, MIR125A, PEG3, SCAF1, TFPT, A1BG, ACPT, AKT1S1, AP2A1, AURKCC, BRCC8, BRSK1, C19orf48, CD33, CHMP2A, CLEC11A, COX6B2, FCAR, FPR1, FPR2, HAS1, HSPBP1, IL11, IL4I1, IRF3, ISOC2, KIR2DL1, KIR2DL3, KIR2DS4, KIR3DL1, KIR3DL2, KLK1, KLK12, KLK13, KLK14, KLK15, KLK2, KLK3, KLK6, KLK8, KLK9, KLKP1, | 9,118,951 |</p>
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                      8T2
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9q22.2
10p15.3 - p11.1
10q11.1 - q11.21
10q11.22 - q26.3
17q11 - q11.1

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CN Loss
CN Loss
CN Loss
CN Loss

8T1
17T1
8T2
8T2
8T2

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<td>BRCA2, CDX2, FLT3, FOXO1, HSPI1, LATS2, LCP1, LHFP, PDX1, STARD13, ZMYM2, AKAP11, ALOX5AP, ATP12A, ATP8A2, C1QTNF9, C1QTNF9B, CCNA1, CDK8, CENPI, CPB2, DCLK1, DNAJC15, ELF1, EPSTI1, ESD, FAM48A, FGF9, FLT1, GJA3, GJB2, GJB6, GTF3A, HMGBl, HTR2A, IFT88, IL17D, KBTBD7, KL, LNX2, MIPEP, MHPHOSPH8, MTUS2, NBEA, PARP4, PDS5B, POSTN, RASL11A, RFC3, RNF17, RNF6, RXFP2, SACS, SAP18, SMAD9, SPATA13, SPG20, TNFRSF19, TNFSF11, TPT1, TRPC4, TSC22D1, WASF3, XPO4, ZDHHC20</td>
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<td>CN Loss</td>
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13q33.1  -  q34  CN Loss  10T1  ANKR1D10, ARGLU1, ARHGEF7, ATP11A, CDC16, COL4A1, COL4A2, CUL4A, EFNB2, F10, F7, GAS6, GRK1, INGI, IRS2, LAMPI, LIG4, MCF2L, PROZ, RAB20, RASA3, SOX1, TFDP1, SUGT1, TBC1D4, THSD1, TNFSF13B  11,043,744

13q33.1  homozygous  8T2,  10T1  371,574

13q33.2  CN Loss  19T1  723,561

13q34  CN Loss  19T1  ATP11A, CDC16, CUL4A, F10, F7, GAS6, GRK1, LAMPI, MCF2L, PROZ, RASA3, SOX1, TFDP1  2,370,496

14q32.2  CN Loss  19T1  BCL11B, SETD3  294,776

15q11.1  -  q26.3  CN Loss  8T2  ADAM10, BLM, BUB1B, C15orf55, CASC5, COP52, CTSH, GCNT3, IGFIR, ITGAI1, KIAA0101, MAPK6, NEIL1, NTRK3, PKM2, PLCB2, PML, RASGRF1, SHC4, SPINT1, TCFI2, THBS1, TRPM1, TYRO3, UBE3A, ACTC1, ADAMTS17, ADAMTS7, ADAMTS13, AEN, AKAP13, ALDH1A2, ALDH1A3, ANP32A, ANPEP, ANXA2, APBA2, APH1B, AQP9, ARHGEF11A, ARID3B, ARH1, ARNT2, ARPP19, ATP10A, AVEN, B2M, BCL2A1, BCL2L10, BMF, BNIP2, C15orf42, C15orf48, CA12, CALML4, CAPN3, CASC4, CCNB2, CCNBPI, CCGP1, CD276, CHAC1, CHD2, CHP, CHRFA7A, CHRFA7B,  81,991,289
CHRNA3, CHRNA5, CHRNA7, CHRNB4, CHSY1, CIB1, CIB2, CKMT1A, CKMT1B, CLK3, COX5A, CPEB1, CRABP1, CRTC3, CSK, CSNK1G1, CSPG4, CT62, CYFIP1, CYP11A1, CYP19A1, CYP1A1, CYP1A2, DAPK2, DENND4A, DET1, DLL4, DNAJA4, DNAJC17, DPP8, DTWD1, DUOX1, DUOX2, DUOX1A1, DUOX2A, DUT, DXY1C1, EID1, EIF2AK4, FAM82A2, FAN1, FANCI, FBNI, FEM1B, FES, FGFI7, FMN1, FOXB1, FURIN, GABRB3, GLCE, GLDN, GN5B, GREM1, HAPLN3, HDC, HERC1, HERC2, HEXA, HMGA20A, IDH2, IGDC4, IL16, IMP3, INO80, IQGAP1, IREB2, ISG20, ITPKA, KIAA1199, KIF23, KLF13, LARP6, LDHAL6B, LINGO1, LMAN1L, LOX1L, LTK, MAN2C1, MAP2K1, MAPKBP1, MEF2A, MEIS2, MESDC2, MESP2, MEX3B, MGE8, MIR1233-1, MIR184, MIR211, MIR9-3, MIRF4L1, MYO5A, NDN, DNFL2, NEDD4, NEO1, NIP2, NOX5, NR2E3, NR2F2, NRG4, NUSAP1, OCA2, OIP5, ONECUT1, PAK6, PAQR5, PAR1, PCSK6, PDCD7, PDLA3, P15, P1F1, P1GB, POLG, PP1B, PPP1R14D, PRC1, PTPN9, RAB11A, RAB27A, RAB8B, RAD51, RASSGR1, RASL12, RCN2, RGM1, RF111, RORA, RPLP1, RPS27L, SCG3, SCG5, SEISBP2L, SELS, SEMA6D, S18N3, SKOR1, SLCL2A6, SLC2A8A1, SLC30A4, SLC30A31, SMAD3, SMAD6, SNAP23, SNRPN, SNURF, SNX1, SORD, SPRED1, ST20, ST8SIA2, STARD9, STOML1, STOML2, TCF4, TLE3, TLE2, TMOD2, TMOD3, TNFAP8L3, TP53BP1, TPM1, TRP17, TTBK2,UBE2Q2, ULK3, UNC45A, USP3, USP8, VPS39, WDR76, ZFAND6, ZNF280D, ZNF592

17p13.1  CN Loss  6T3  p53, SHBG, TP53, WRAP53, ATP1B2, CD68, CHRNB1, EFNB3, EIF4A1, FGF11, FXR2, KCTD11, POLR2A, SAT2, SENP3, TNFSF12, TNFSF12-TNFSF13, TNFSF13, TNK1, ZBTB4  423,831

18p11.32  CN Loss  19T1  METTL4, NDC80  163,566

18p11.32 - p11.31  CN Loss  19T1  EMIL1N2, MYL12A, MYOM1, TGIFI  1,060,976

18q12.2  CN Loss  19T1  DCC, MALAT1, MAPK4, MBD2, SMAD2, SMAD4, ATP8B1, C18orf26, CCBE1, CCDC68, ELAC1, GRP, IER3P1, LIPG, LMAN1, MBD1, ME2, MEX3C, MIR122, NEDD4L, ONECUT2, PIAS2, PIK3C3, PMAIP1, POLI, PSTPIP2, RAB27B, RIT2, SETBP1, SLC14A1, SMAD7, TCF4, TXN1L  18,329,050

18q21.32  CN Loss  19T1  SERPINB8  325,592

18q21.33 - q22.1  CN Loss  19T1  SERPINB8  1,566,817
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<th>Event</th>
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<th>Log_2 Avg. Ratio</th>
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<td>AURKA, ATP5E, BMP7, C2orf85, CDH4, CSTF1, CTCFL, CTSZ, EDN3, GNAS, MIR296, PCK1, PHACTR3, PMEP1, PPP1R3D, RAB22A, TFAP2C, TH1L, TUBB1, ZBP1</td>
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Appendix 3: Copy number variations (CNVs) identified in SFTs, sorted according to their chromosomal location. The number of SFT cases with these CNVs is indicated.

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<th>Locus</th>
<th>Event</th>
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